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# CLINICAL CHEMISTRY

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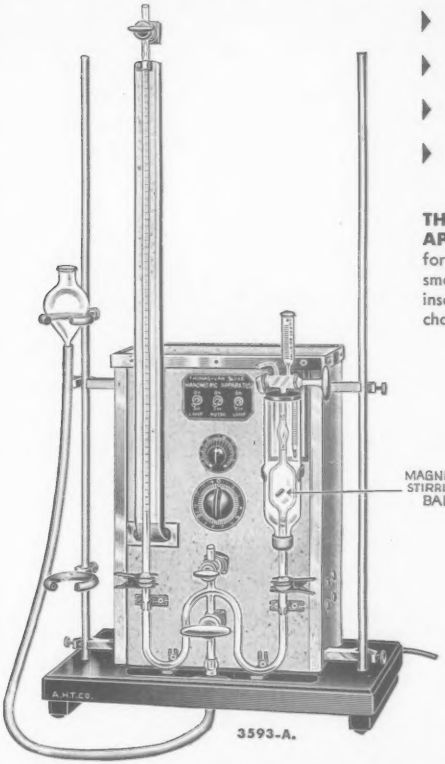

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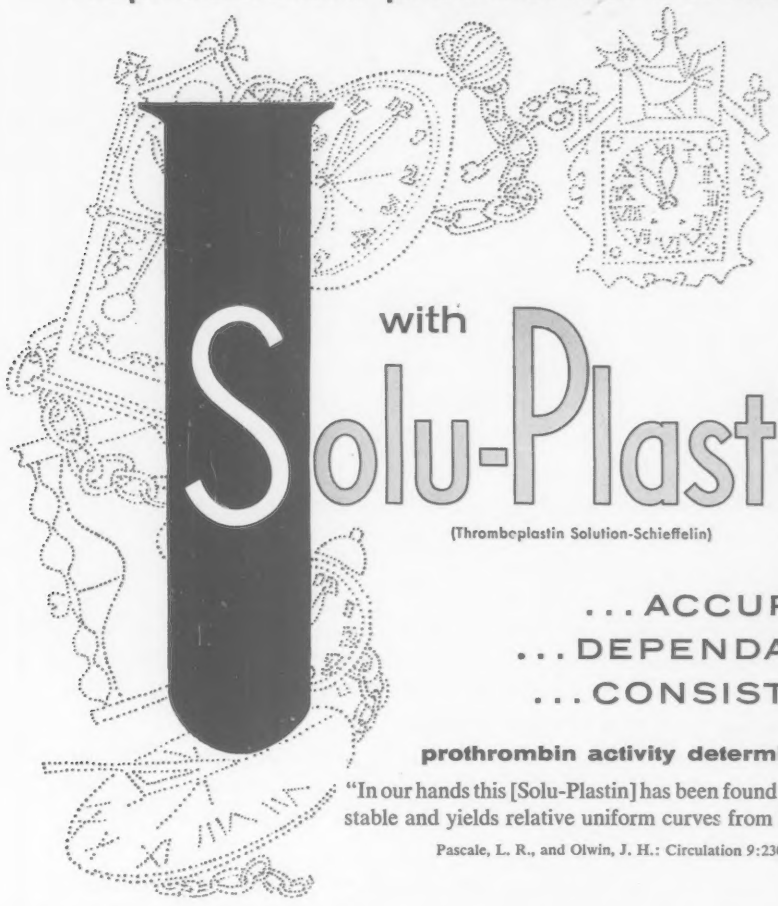

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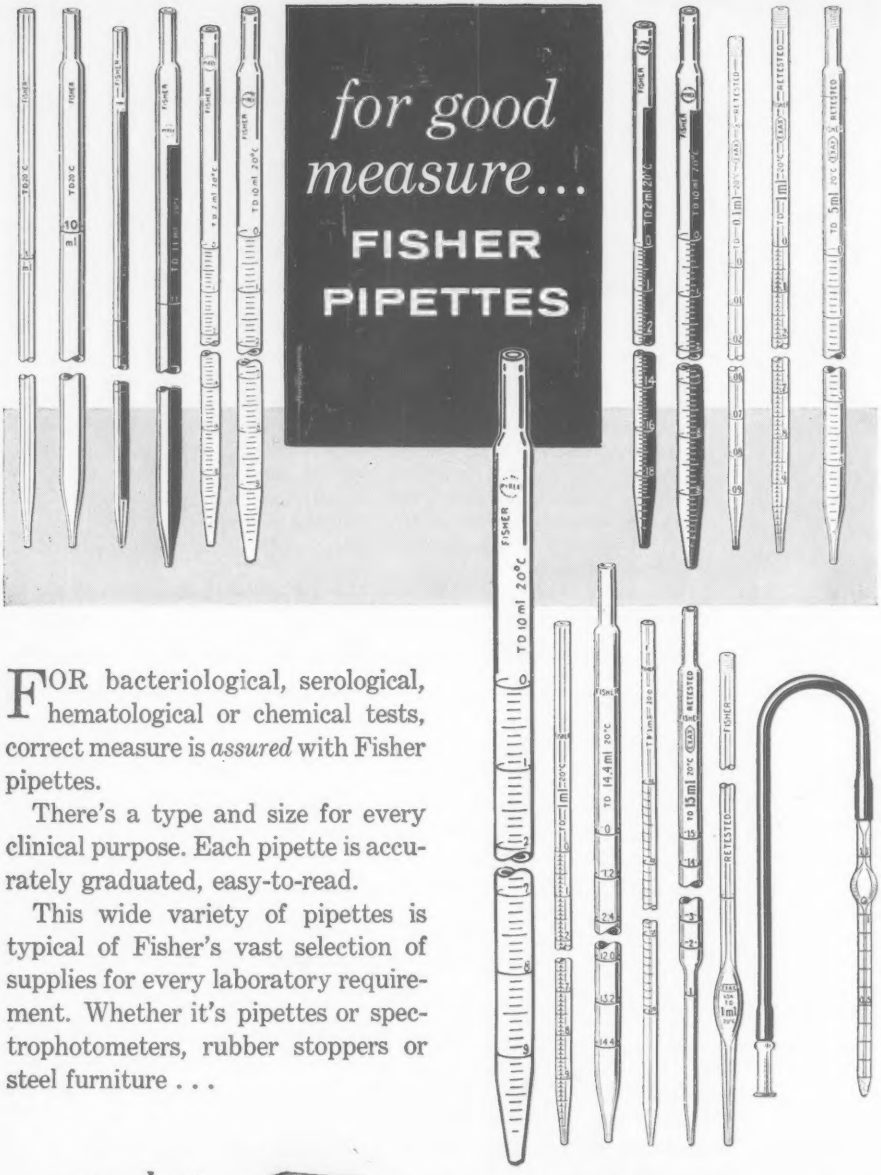


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# CLINICAL CHEMISTRY

*Journal of the American Association of Clinical Chemists*

VOLUME 1 APRIL 1955 NUMBER 2

# Serum Lipoproteins

## Chemical and Enzymatic Studies

*Ray K. Brown, Louis S. DeLalla, and Dorothy L. Kauffman*

ALTERATION IN BLOOD LIPIDS is associated with a wide variety of diseases, among them xanthomatosis and atherosclerosis. Excessive loss of carbohydrate or protein from the body, as in diabetes, nephroses, multiple myeloma, or chronic hemorrhage, is frequently accompanied by blood-lipid derangement. Studies of the structure and linkages which bind lipids to protein molecules and of the mechanisms controlling their concentration may give clues to the varied diseases associated with deranged lipid metabolism.

The unusual solubility properties of the serum lipids have been attributed to lipid protein complexes for over half a century.[1] Macheboeuf was among the early workers to isolate a lipoprotein and determine its composition.[2] Serum lipoproteins are usually divided in those of high density, $\alpha_1$, and $\alpha_2$ lipoproteins; those of low density, the $\beta$ lipoprotein described by Oncley, Gurd, and Melin;[3] and larger lipoproteins of Sf 10 and greater as described by Lindgren, Elliott, and Gofman.[4] There is considerable evidence that several of these lipoproteins consist of more than one macromolecular entity. The fractionation experiments of Batchelor[5] and ultracentrifuge studies of Boyle *et al.*[6] indicate the heterogeneity of $\alpha_1$ lipoproteins, the immunochemical investigations of Gitlin[7] suggest the heterogeneity of the $\beta$ lipoproteins,* and the electron microscope studies of Beischer[8] on Sf 30 lipoproteins indicate that these substances are not homogeneous. Although chylomicrons are probably heterogeneous aggregates of lipids, it is possible that subfractions of

From the Division of Laboratories and Research, New York State Department of Health, Albany, N. Y.

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* Recent gradient tube experiments of Oncley and Mannick also support this view.

smaller lipoproteins are homogeneous molecular entities. It is only by attempting to further purify these lipoproteins that an answer to these questions can be found.

## PREPARATION OF LIPOPROTEINS

### Preparation of $\alpha_2$ Lipoproteins

When plasma is fractionated by Cohn's method,[9] most of the low-density lipoproteins and considerable amounts of $\alpha_2$ lipoprotein fall into fraction III-O. This fraction can be further purified by dialyzing the freshly prepared paste twice at $-5°$ C. for twenty-four hours against 30 volumes of a 1.65 M solution in sodium chloride. The alcohol is thus removed and the paste brought into solution at a solvent density of 1.06. This solution is ultracentrifuged for eighteen hours at 97,720 G and the floating low-density lipoprotein concentrate is sliced from the top of the tube by means of a sharp blade in a rigid holder. This material is saved for further purification. The solution immediately below the low-density lipoproteins is discarded and the proteins in the bottom of the tube are decanted from the pellicle. These proteins are fractionated with neutral ammonium sulfate at 0°. The fraction precipitating between 33- and 42-per-cent saturation is dissolved in a minimal amount of $H_2O$, brought to a solvent density of 1.20, and ultracentrifuged as before. The $\alpha_2$ lipoproteins sliced from the top are ultracentrifugally homogeneous at a density of 1.20 and the solution has a single electrophoretic peak with a mobility of $-2.56 \times 10^{-5}$ in barbital buffer, *p*H 8.6, $\Gamma/2 = .1$. Their minimum solubility lies near *p*H 4.9 (Fig. 1). However, when these lipoproteins are tested immunochemically on an agar plate, they still give faint lines with anticrystalline albumin, antigamma globulin, and anticrystalline glycoprotein sera.[10]

### Preparation of $\beta$ Lipoproteins

The low-density lipoprotein concentrate from fraction III-O may be further purified by diluting it with 3.07 volumes of physiological saline to a density of 1.02 and ultracentrifuging as before. This separation is less sharp than that at density 1.06. The lipoproteins of Sf 10 and greater are sliced from the top of the tube. The $\beta$ lipoproteins are more than 95 per cent homogeneous electrophoretically. They will not form a boundary in the descending limb due, presumably, to density differences. The hexose content of this material is typical of that of most proteins, about 0.4 per cent.

If the $\beta$ lipoproteins are to be preserved for any appreciable time, the *p*H should be adjusted to 6.5 and Merthiolate, .01%, added. This *p*H

range is similar to that of maximum stability for serum albumin and gamma globulin. Beta lipoproteins stored for a few weeks below *p*H 6.0 form very turbid solutions. The turbidity is not affected by ethylene diamine tetracetate or *p*H shift.

Beta lipoproteins prepared as described have remained stable without turbidity change for several months at 0° and have not, as yet, permitted any observations on the effects of esterase inhibitors, antioxidants, and chelating agents. Studies of other agents on the stability of beta lipoproteins have been made by Gurd.[11]

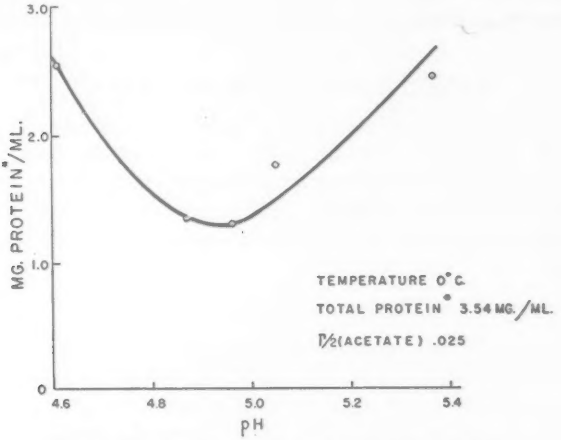


**Fig. 1.** Solubility of $\alpha_2$ lipoprotein at various *p*H's.

### Preparation of $\alpha$ Lipoproteins

A lipoprotein fraction of human plasma, which was 90 per cent homogeneous electrophoretically and ultracentrifugally, was isolated under conditions similar to those used by Cohn and coworkers[12] and by Kominz.[13]

The material used was fraction IV-I paste separated from plasma by Cohn's Method 6. A weighed amount of paste was dissolved in twice its weight of water containing sufficient sodium bicarbonate to raise the *p*H above 6. The addition of water was begun with the paste at $-5°$ and completed at 0°. All subsequent manipulations were carried out at 0°. The solution containing fraction IV-I was dialyzed against distilled water for four or five days. The reddish brown precipitate which formed was removed by centrifugation. The supernatant was adjusted to an

ionic strength of 0.1 with sodium chloride and to *p*H 5.1–5.2 with acetate buffer, *p*H 4.0, $\Gamma/2 = .01$, eighty times concentrated. The resulting suspension was separated by centrifugation and the precipitate was washed once with 0.1 M sodium chloride buffered to *p*H 5.2. The washed precipitate was dissolved in a suitable buffer at approximately neutral *p*H.

This lipoprotein fraction has been partially characterized chemically and its composition resembles closely that of fraction IV-I (1) reported by Rosenberg.[14] It has a protein:cholesterol ratio of 0.6 and a cholesterol:phospholipid ratio of 0.2. A 1% solution has an $S_{20w}$ of 4.5 and an electrophoretic mobility of $-4.8 \times 10^{-5}$ at *p*H 8.6 in .1 M barbital buffer.

## ALTERATION OF LIPOPROTEINS BY ENZYMES

Previous workers have shown that administration of heparin clears the turbidity of alimentary lipemia[15] and causes alterations in the serum lipoproteins[16] as determined ultracentrifugally. Lipoproteins of high Sf are broken into smaller lipoproteins. These alterations may be divided into two major steps, the formation of clearing factor and its action on lipoproteins.

### Formation of Clearing Factor

In the intact animal, injection of many different substances may give rise to clearing activity. All are acidic substances of relatively large molecular weight or salts of these substances. Many are sulfates, although phosphotungstic acid, a substance very different from heparin, has been found by Bragdon and Havel[17] to produce clearing factor in rats.

The recent work of Korn[18] indicates that ammonia extracts of an acetone powder of normal rat heart contain clearing factor. Previous work with heart minces indicated that a component of plasma was necessary for formation of the clearing factor.[19] This action is probably solubilization, which would agree with the results of Nikkilä[20] who found some clearing activity in mortar-ground heart extracts in the absence of plasma, although plasma extracts were more effective. It is inadvisable to use the term precursor for this activity. If clearing factor must be solubilized before it can act under physiologic conditions, a plasma component may be involved. Some clearing activity in normal rat serum has been reported[19] and Jeffries[21] has extended these observations. Anfinsen[22] has suggested that clearing may occur in the tissues and that clearing factor may not normally be in solution. The stability of lipemic serum is in accord with this hypothesis.

### Substrate Specificity

It has been shown that serum containing clearing factor has no greater activity toward $\beta$ naphthyl laurate than preheparin serum from the same animal.[23] Similar results are obtained when $\beta$ carbonaphthoxy choline iodide or $\beta$ naphthyl chloracetate is used as substrate, which indicates that the esterase activity of clearing factor is not responsible for the histochemically demonstrable staining of mast cells with $\beta$ naphthyl chloracetate as observed by Gomori.[24]

Emulsions of most triglycerides of short chain,[25] long chain, or unsaturated fatty acids are cleared by clearing factor. The rate of attack on triglyceride emulsions seems related more to the size of the emulsion than to the triglyceride used. The data in Table 1, which are typical of six experiments, indicate that monopalmitin is also attacked by clearing factor. Korn[18] has shown that lipoproteins are needed before clearing factor made in vitro can attack triglyceride emulsions. Since these experiments were done with clearing factor prepared in vivo, it is uncertain whether lipoprotein components are required for the substrates.

**Table 1.** HYDROLYSIS OF MONOPALMITIN BY CLEARING FACTOR

| | *Fatty acid liberated* ($\mu$) m |
|---|---|
| Preheparin serum | 0.15 |
| Clearing factor serum | 2.7 |

1 ml. of dog serum incubated for 3 hours with 1 ml. of an emulsion in 1.5% gelatin containing 1.5 mg. (45 $\mu$ m) of 1 monopalmitin. With smaller amounts of monopalmitin hydrolysis is complete, indicating that monopalmitin rather than impurities is being attacked.

### Substrate Competition Experiments with Clearing Factor

Although monoglycerides and triglycerides compete with $\beta$ naphthyl laurate for esterase, alimentary lipemic lipoproteins do not unless clearing factor is present. Nor do they compete for choline esterase either in the presence or absence of clearing factor when $\beta$ carbonaphthoxy choline iodide is used as substrate. Since no data are available on the affinity of this enzyme for its substrate, one cannot rule out the possibility that choline esterase plays a part in esteratic breakdown, although the lack of inhibition of clearing by eserine and diisopropyl fluorophosphate (DFP),* $10^{-5}$ M, suggests that it does not. These findings indicate that plasma aliphatic esterase has little to do with the initial reaction of lipoprotein breakdown and that it does play an important part in glyceride hydrolysis.

* We are indebted to Dr. Robert F. Sterner of Merck and Company and to Dr. William H. Summerson of the United States Army Chemical Corps for a supply of DFP.

## ACTION OF INHIBITORS ON THE CLEARING SYSTEM

Previous reports have indicated that clearing factor is inhibited by a high concentration of salt and of protamine. It is not inhibited by most other enzyme poisons.[25] Shore, Nichols, and Freeman[26] have reported that arsenite is an inhibitor. At the concentrations employed, the *p*H is quite alkaline to the *p*H optimum and attempts to titrate the material toward neutrality cause precipitation of arsenous oxide. Under these conditions, no inhibition occurs. DFP is a fairly general inhibitor of hydrolytic enzymes. It inhibits clearing factor at low concentrations

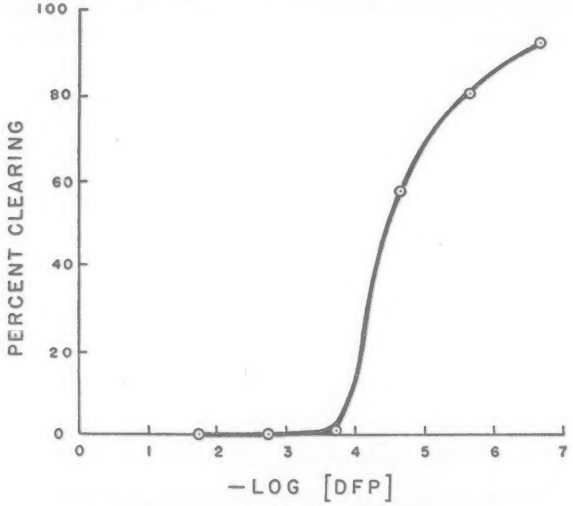


**Fig. 2.** Inhibition of clearing by DFP.*

(Fig. 2), which suggests that clearing factor may be a hydrolytic enzyme. In a crude way it gives an indication of its maximum concentration. If the concentration of clearing factor is assumed to be twice that of the DFP giving a 50 per cent inhibition, its concentration must be $3 \times 10^{-5}$ M or less. However, it should be emphasized that the concentration

* Freshly opened DFP (.036 ml.) was emulsified in 2.0 ml. of *p*H 7.7 $\Gamma/2$ = .1 phosphate buffer. A series of 1:10 dilutions was made at once and .42 ml. of each was mixed immediately with 1.4 ml. of dog plasma containing clearing factor. They were allowed to stand, with occasional agitation, at room temperature for one hour and dialyzed against 4 L of saline, buffered with phosphate to *p*H 7,[34] in a rocking dialyzer for one hour. A control sample was treated similarly, except for DFP addition, and was dialyzed in a separate arm of the rocker. Clearing was observed in a tube containing 0.26 ml. of each sample, 0.2 ml. of dog alimentary lipemic serum, saline, phosphate buffer, and EDTA as described previously.

of clearing factor may be many times smaller since DFP may be lost from the system by spontaneous hydrolysis or by coupling to other proteins. There is no assurance that a 1:1 enzyme:inhibitor ratio will hold for clearing factor as it does for chymotrypsin.[27] In a typical experiment, corrected by these assumptions, 120 moles of fatty acid were liberated by one mole of clearing factor, a clear indication of the enzymatic nature of clearing factor.

Table 2 indicates that DFP inhibits clearing factor itself rather than any of the other components of the clearing mechanism. The addition of normal serum to a DFP-inhibited system does not remove the inhibition. Controls in which clearing factor containing serum was added show the expected amount of clearing which rules out the possibility that

Table 2. Effect of DFP on Clearing

| | *Decrease in optical density at 500 mμ in 1 hr.* |
|---|---|
| 1. Clearing factor | .194 |
| 2. DFP clearing factor | .010 |
| 3. DFP clearing factor + preheparin serum | .021 |
| 4. DFP clearing factor + clearing factor | .205 |

Dog serum heparinized *in vivo*, 0.2 ml. per determination, was used as clearing factor. DFP clearing factor was $2 \times 10^{-4}M$ in DFP. Ethylene diamine tetracetate (EDTA)-phosphate, 0.2 ml., was added to each tube, the volume was brought to 0.9 ml. with saline, and 0.1 ml. of lipemic dog serum was added. The tubes were incubated at 37°C.

free DFP remains and inhibits the added normal serum. Lipoproteins treated with DFP are cleared by clearing factor.

Surface active agents are another group of substances which inhibit clearing. The effect of Triton WR 1339 on lipoproteins had been described.[25] Lipoproteins treated with Triton and washed are not cleared. They do not inhibit the clearing of control lipoproteins which were treated similarly but with saline instead of Triton. This suggests that Triton inhibits clearing by altering the lipoprotein. Nikkilä's report[20] that Tween inhibits clearing has been confirmed. Cholate is also an inhibitor. It is probable, although not yet proved, that Tween and cholate act by the same mechanism as Triton.

Gordon *et al.*[28] have described another type of clearing inhibition in which fatty acid is added to the crude clearing factor in excess of the amount needed to saturate albumin. The addition of recrystallized serum albumin restores the activity. Robinson and French[29] have reported that serum albumin added to chyle and clearing factor brings about additional clearing.

Fatty acid or glycerol formation, ultracentrifugal alteration, and

optical density change have been used frequently to assay clearing. In crude systems, where two methods have been used simultaneously, good agreement has usually been obtained. However, one should be cautious about interpreting the phenomenon in purified systems or in systems containing unusual substances. The clearing observed when chylomicrons are placed in 40% glycerol appears to be due merely to the approach of solvent and solute refractive index since glycine or sucrose solutions of the same refractive index cause similar clearing. Indeed, the experiment is like that of the magician who makes a glass ball disappear in a glass of so-called water, only repeated on a micro scale.

The determination of glycerol liberation in purified systems may not give reliable results unless care is taken to control carefully the concentration of aliphatic esterase since this substance plays a part in the breakdown of glycerides. Fatty acid determination is open to similar objections, although in properly controlled experiments most of the fatty acid is a result of clearing factor activity.

### Action of Other Enzymes on Lipoproteins

Preparations containing pancreatic and gastric lipases attack lipoproteins and cause clearing, fatty acid formation, and often an increase in $\alpha$ lipoprotein concentration. Castor bean lipase has no effect. Although the clearing activity in plasma extracts of gastric lipase fractionates in a manner similar to clearing factor and is inhibited by salt and protamine, there is considerable evidence that they are not identical. Clearing factor made in vivo requires nonlipoprotein components during its course of activity, may be complexed onto coconut oil emulsions, and has no esterase activity with $\beta$ naphthyl laurate as substrate. Extracts containing gastric lipase do not possess these properties. The course of clearing with gastric lipase often shows an initial lag period whose length is a function of the amount of lipase used.[30] Gastric and pancreatic lipase possess almost full activity at DFP levels one thousand times those that cause 50 per cent clearing factor inhibition. The substance causing clearing may be solubilized from mouse pyloric mucosa by $\alpha$ lipoproteins or by 0.025 M ammonium hydroxide. This suggests that $\alpha$ lipoprotein may merely serve as an extractant, perhaps by forming an enzyme–substrate analogue combination.

Rosenberg[31] has shown that proteolytic digestion of lipoproteins must proceed to a considerable extent before ultracentrifugal changes occur. Studies by Williams[32] on the action of *Clostridium welchii* lecithinase on lipoproteins indicate that the optical density of lipoprotein solutions, pretreated with lecithinase, is not affected by clearing factor.

### Relationship of Clearing Factor to Plasma Esterases

It is apparent that clearing factor is neither of the enzymes that account for most of the esterase activity of plasma. Plasma choline esterase is inhibited by low concentrations of DFP, clearing factor is not. Grossman[33] has shown that clearing is not appreciably inhibited by physostigmine at levels that inhibit choline esterase, as confirmed here. Plasma aliphatic esterase is not inhibited by protamine in concentrations that inhibit clearing factor and is only about 50 per cent inhibited by DFP in concentrations that completely inhibit clearing factor. Both plasma aliphatic esterase and choline esterase attack $\beta$ naphthyl laurate. Since no increase in $\beta$ naphthyl laurate esterase activity occurs during formation of clearing factor, it cannot be either of the plasma esterases. As yet no inhibitor of plasma aliphatic esterase has been found which does not also inhibit clearing factor.

Whether appreciable amounts of specific lipase exist in plasma is uncertain. Both plasma choline esterase and plasma aliphatic esterase attack triglycerides. Thus far, attempts to use substances that partially inhibit or partially activate them have given equivocal results.

## SUMMARY

The larger lipoproteins of Sf greater than 10 also contain triglycerides. The action of lipases on these lipoproteins, either heparin-induced clearing factor, pancreatic lipase, or gastric lipase, yields fatty acids, glycerol, and smaller lipoproteins. Crude clearing factor attacks monoglycerides and triglycerides and is inactive against several esters which plasma esterases attack. Albumin, plasma esterases, and probably other components are needed for optimal clearing factor activity. Low concentrations of diisopropyl fluorophosphate (DFP), a substance inhibiting most hydrolytic enzymes, inactivate crude clearing factor. Stoicheometric calculations made from this experiment indicate further the enzymatic nature of clearing factor. Evidence that clearing factor is different from other lipases and from esterases found in plasma is presented.

Studies with purified lipoproteins are needed to determine what alterations, if any, occur in smaller lipoproteins. The separation and purification of lipoproteins by chemical and ultracentrifugal means are described. Whole human plasma separated by Cohn's methods 6 and 9 serves as starting material for the preparation of $\alpha_1$, $\alpha_2$, $\beta$, and Sf 10 and greater lipoproteins.

Studies of lipoproteins may give clues to the varied diseases associated with deranged lipid metabolism, such as xanthomatosis and atherosclerosis.

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# Recent Studies of Fat Digestion and Absorption

*Raymond Reiser*

THE MECHANISM WHEREBY water-insoluble fat can pass through the intestinal epithelium and into the blood has been a very intriguing as well as a most refractory problem. Obviously, the fat must either pass as particles or it must be solubilized, and various theories have been devised based on both possibilities.

Most studies on the degree of hydrolysis of fat during digestion have been undertaken to obtain clues of the mechanism of the absorption of fat. If it could be demonstrated, for example, that fat is always completely hydrolyzed in the lumen of the intestine, it would be necessary to explain the absorption of fatty acid but not of glycerides. On the other hand, if it could be demonstrated that hydrolysis is not complete, then, *ipso facto*, glycerides must be absorbed and the mechanism of their absorption must be explained.

Up to the advent of the use of isotopes as tracers, no experiment was designed which could demonstrate unequivocally whether or not hydrolysis is complete or whether glycerides are absorbed.

## IN VITRO DIGESTION

"Modern" studies on the mechanism of digestion of fat, and the action of lipases, may be said to have originated with the work of Artom and Reale in 1935 (1). These workers fractionated the products of in vitro pancreatic lipase digestion in alcohol–water and acetone–water solutions. They found that, contrary to opinion up to that time, lipase action produced little if any glycerol but mainly mono- and di-glycerides.

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It was not until ten years later that Frazer and Sammons independently rediscovered this phenomenon and announced that during the first four hours of in vitro fat digestion little if any glycerol is produced and that considerable quantities of monoglycerides are produced (2).

Two years after Frazer's rediscovery of the limited hydrolytic capacity of pancreatic lipase on monoglycerides, Desnuelle and his coworkers (3, 4) verified the observation of these in vitro studies. These authors showed conclusively that pancreatic extracts produce diglycerides readily, that the production of monoglycerides requires more time and the presence of calcium ions, and that the digestion of monoglycerides requires "a large aqueous phase rich in bile salts" and much more time.

Schønheyder and Volqvartz have made confirmatory studies demonstrating the increasing difficulty of hydrolysis with increase in number of free hydroxyl groups (6). These workers found that the action of liver esterases on tripropionin produces the diglyceride easily, the monoglyceride with more difficulty, and free glycerol only after prolonged time. Under their conditions pancreatic lipase could hydrolyze the substrate only to the diglyceride.

In a study to determine the most susceptible glyceride ester linkages Desnuelle, Naudet, and Rouzier have reported (5) that the proportions of 1- and 2-monoglycerides formed are 2:1, an observation which, if confirmed, would indicate that there is no specificity for the 1- or the 2-positions.

In 1952 (7) and in 1954 (8) Borgström reported in vitro studies in which base exchange resins and silicic acid chromatography were utilized to separate the products of digestion, and $C^{14}$ labeled fatty acids to trace the course of the reactions. Borgström reported that after one hour of digestion with pancreatic lipase the digestion mixture contained considerable quantities of tri- and mono-glycerides but that there was no free glycerol. In 24 hours digestion was complete (7).

In Borgström's studies (8) labeled fatty acids were added to digestion mixtures containing triglycerides. It was found that an equilibrium was reached between the free fatty acids and the glyceride fatty acids. At equilibrium the specific activity of the glyceride fatty acids, however, were less than that of the free fatty acids. Separation of the glycerides by silicic acid chromatography, and direct measurement of the specific activities of their constituent fatty acids showed that the triglycerides had about two thirds of the activity of the free acids, the diglycerides about one half, and the monoglycerides about one tenth.

Borgström explained these results according to the scheme shown in Fig. 1. If one assumes that only the 1- and 3-positions are in equilib-

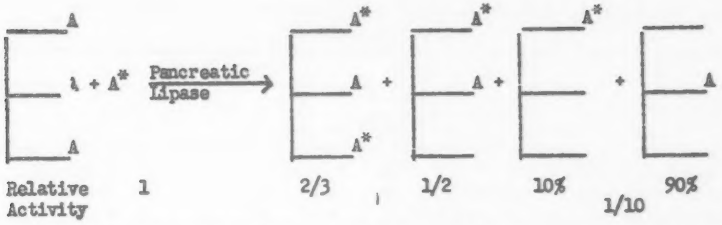



**Fig. 1.** The products of the digestion of triglycerides in the presence of labeled free fatty acid. From Borgstrom. (8)

rium with the free fatty acids the data are explainable. The fatty acid on the 1- and 3- positions would then have the same activity as the free fatty acids but those on the 2-position would remain inert. This hypothesis would require that 90 per cent of the monoglycerides are of the 2-isomer. To the contrary, Mattson, Benedict, and Beck have recently found by chemical analysis that only the 1- monoglyceride is formed in vitro but that 2- monoglycerides are the predominant monoglyceride formed in in vivo digestion (9).[1]

To sum up the results of in vitro studies beginning with the 1935 work of Artom and Reale, it has become increasingly evident that diglycerides are readily produced by in vitro lipase action, that monoglycerides are produced with more difficulty and require more time and more exacting conditions, and that the production of glycerol is slow and refractory.

The in vitro studies are conflicting with respect to the relative specificity of the 1-, 2-, and 3- positions. Desnuelle reported that twice as much 1- as 2- monoglycerides are formed, indicating equal specificity (5). Borgström adducted evidence to show that the 2-isomer is the predominant one formed (8), while Mattson, Benedict, and Beck found that in vitro pancreatic digestion produced mainly the 1-isomer (9).[1]

## IN VIVO DIGESTION

The past two years have produced a number of important studies on in vivo lipolysis (Table 1). Desnuelle and Constantin (10) examined the intraluminary lipides three hours after olive oil ingestion and found that it consisted of approximately 50 per cent triglycerides, 21 per cent diglycerides, 5 per cent monoglycerides, and 16–24 per cent free fatty acids.

[1] In a recent communication, Dr. Mattson informed this author that in new studies by his group, the 2-monoglycerides have been identified in the products of in vitro digestion.

**Table 1.** LIPIDE ANALYSES OF THE INTESTINAL CONTENTS AFTER TRIGLYCERIDE DIGESTION

| | *Glyceride species* | | | | |
|---|---|---|---|---|---|
| | *Tri-* (%) | *Di-* (%) | *Mono-* | | *FFA* (%) |
| | | | *1-* (%) | *2-* (%) | |
| Desnuelle *et al.* (10) | 50 | 21 | 5 | | 16–24 |
| Mattson *et al.* (11) | 41 | 36 | 3.2 | 4.3 | 15.5 |
| Mattson *et al.* (9) | 60–70 | .. | 4–7 | 4–7 | 25 |
| Kuhrt *et al.* (13) | .. | .. | 37.6 | | .. |
| | | | 50.0 | | .. |
| Harris *et al.* (14) | .. | .. | 2.5 | 2.5 | 2–3 times the monoglycerides |

Mattson, Benedict, Martin, and Beck (11) found that three hours after feeding triglyceride the lipides of the intestine consisted of 41 per cent triglyceride, 36 per cent diglyceride, 4.3 per cent 2-monoglyceride, 3.2 per cent 1-monoglyceride, and 15.5 per cent free fatty acid. These equal proportions of the 1- and 2- monoglycerides were in contrast to their in vitro findings (9)[1] but in confirmation of Desnuelle. That equal proportions do not mean equal susceptibility of the 1- and 2- positions to hydrolysis however, was demonstrated by an experiment with the use of 2-oleyl-dipalmitin (Fig. 2). After the ingestion of this compound by the rat, analyses of the intestinal lipides showed that there were about equal proportions of 1- and 2- monoglycerides. The iodine value of the mixed monoglycerides, however, was 64, and of the free fatty acids only 5. This indicates that the palmitic acid radicles must first have been hydrolyzed and half the resultant 2-monoolein rearranged to form 1-monoolein.

More recently Mattson, Benedict, and Beck (9) examined the lipides of the intestinal contents after the ingestion of cottonseed oil, of cottonseed oil with various proportions of 1-monoglyceride, cottonseed oil with varying amounts of 2-monoglyceride, and cottonseed oil with varying amounts of free fatty acids. Surprisingly, after 3 hours of digestion of the oil, alone or with any amount of either monoglyceride, the lipides of the digestion mixture had the same composition: 60–70 per cent triglycerides plus diglycerides, 4–7 per cent 1-monoglycerides, and about 25 per cent free fatty acids. When fed with free fatty acids, there was no 2-monoglyceride found, although there were 6–15 per cent 1-monoglycerides.

In an undetailed experiment, these authors reported that they fed 1-monoglyceride alone and found no 2-monoglyceride, diglyceride, or triglyceride.

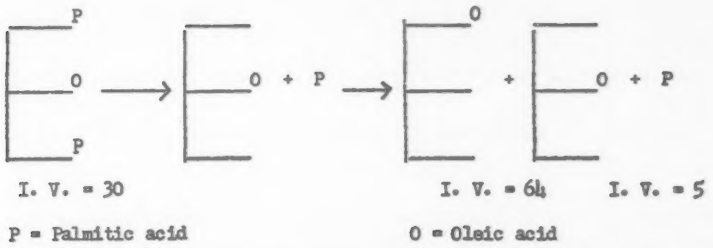



**Fig. 2.** Evidence that 2-monoglycerides are the product of in vivo fat digestion, followed by rearrangement to the 1-isomer from Mattson *et al.* (11)

The observations of Mattson, Benedict, and Beck, upon feeding monoglycerides alone, or triglycerides plus free fatty acids, are in strong contrast to a recent report by Borgström (12). Upon feeding olive oil with labeled palmitic acid, or monoolein with labeled oleic and palmitic acid, this worker found the label in mono-, di- and tri-glycerides, all apparently produced during digestion.

The resolution of these conflicting findings is not apparent at this time. Mattson's results indicating that the 2- position cannot be esterified during digestion is in support of older studies by Artom and Reale (13). Beginning with glycerol and fatty acids these workers found it much more difficult to esterify the 2- position than the 1- or 3-.

There have been two recent studies on in vivo lipolysis in humans. Kuhrt, Welch, Blum, Perry, Weber, and Nasset (14) examined the lipides of the intestinal contents of 2 humans after the ingestion of oil. They found the lipides to contain 37.6 per cent and 50 per cent monoglycerides in the 2 subjects. This figure is five to ten times as high as that found by others.

Recently Harris, Chamberlain, and Benedict (15) reported finding 4–11 per cent of monoglycerides, evenly divided between the 1- and 2-isomers, in the intestines of 11 human subjects after fat meals. The free fatty acid content was two to three times that of the monoglycerides. This is similar to the results obtained by Mattson *et al.* with rats (11).

Gidez and Karnovsky, in a private communication,[2] state that they have measured the degree of hydrolysis by comparing the rate of $C^{14}O_2$ excretion after the ingestion of free $C^{14}$-labeled glycerol and triolein, $C^{14}$-labeled in the glycerol. They found that about 12 per cent glycerol was hydrolyzed during the first hour, 14 per cent during the first two

[2] Private communication. L. I. Gidez, Brookhaven National Laboratory, Upton L. I., New York, M. L. Karnovsky, Department Biological Chemistry, Harvard Medical School, Boston, Mass.

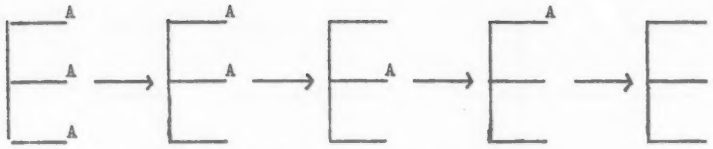


Fig. 3. Probable sequence of pancreatic lipolysis.

hours, 52 per cent at three hours and 97 per cent at eight hours. Although they have found some free labeled glycerol in the lumen of the intestine during fat digestion they believe intralumenary hydrolysis to glycerol to be slight and that the hydrolysis somehow takes place during transit of the ingested lipide from the mucosal cells to lymph.

In summary we can say that the bulk of both in vitro and in vivo experiments indicates that pancreatic lipolysis proceeds from triglycerides to glycerol stepwise with increasing difficulty, that the 1- and 3- positions are first split, that the 2- monoglycerides probably rearrange to the 1-isomer and that it is the 1-isomer that is finally hydrolyzed (Fig. 3).

## ABSORPTION

Unfortunately our new knowledge of the steps in lipolysis allow us to draw no conclusions as to which species of glycerides are absorbed. The fact that pancreatic digestion is capable of hydrolyzing glycerides completely to fatty acids and glycerol does not mean that such happens and that no glycerides are absorbed. On the contrary, during the past two years unequivocal evidence has been obtained that the larger part of lipide glycerol is absorbed as glycerides. It remains to be determined whether all the lipide glycerol is so absorbed and whether it is in the form of only monoglycerides, or mixtures of monoglycerides with di- and triglycerides.

In 1951 Favarger, Collet, and Cherbuliez fed a mixture of triglycerides and deuterium-labeled glycerol to rats and examined the lymph glycerides for the labeled glycerol (16). They found that the glycerol was incorporated to the extent of only about 1.5–4.5 per cent into the resynthesized lipides and concluded that very little hydrolyses must take place and that 90 per cent of the fat must have been absorbed as mono-, di- and tri-glycerides (Fig. 4).

The rationale behind this experiment was that if hydrolysis were complete, the hydrolyzed glycerol would mix with the labeled free glycerol and the specific activity of the resynthesized fat, as it appears in the lymph, would reflect the degree of hydrolysis. Unfortunately their as-

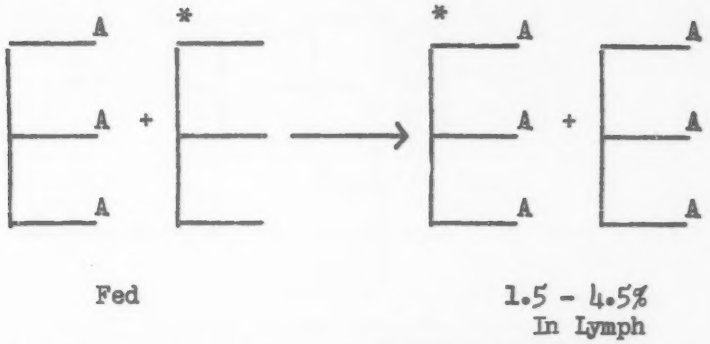



**Fig. 4.** Incorporation of ingested glycerol into lymph glycerides. From Favarger, Collet, and Cherbuliez. (16)

sumption was wrong. As it was later demonstrated, and several times confirmed, free glycerol in the intestinal lumen is not incorporated into resynthesized glycerides. Favarger's failure to incorporate labeled free glycerol into lymph glyceride need not then be due to lack of hydrolysis.

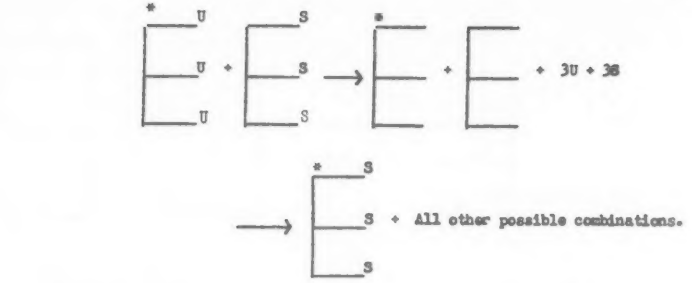
In 1952 we reported our first studies with glycerides labeled in both the glycerol and fatty acid moieties (17). In the first experiments rats were fed conjugated trilinolein in which the glycerol was labeled with $C^{14}$. Sixty per cent of the labeled glycerol was found in the lymph glycerides. (That is, after correcting for biological dilution, the lymph glycerol had a specific activity of 60 per cent as compared to that fed.)[3] It is possible either that the 60 per cent had been absorbed as glycerides or that the glycerides had been hydrolyzed and the labeled glycerol reutilized.

[3] In these calculations, in order to correct glycerol activity for biological dilution, it is assumed that ingested fatty acids undergo no metabolism before their appearance in the lymph and that the biological dilution of the glycerol is the same as that of the fatty acid. The following equation, therefore, may be used to determine the percentage of activity in the glycerol of the lymph or mucosa lipides as compared to the activity of the glycerol fed, corrected for biological dilution.

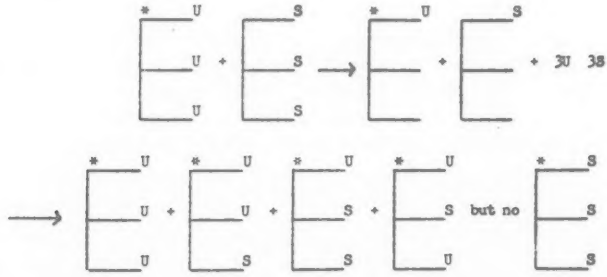
$$R = \frac{AF}{Ar} \times \frac{Gr}{Gf} \times 100$$

$R$ = percentage of activity of recovered glycerol as compared to the glycerol fed;
$Af$ = activity of fatty acid fed;
$Ar$ = activity of fatty acid recovered;
$Gf$ = activity of glycerol fed;
$Ar$ = activity of glycerol recovered.

One may then calculate the percentage of glycerol activity lost (or, the percentage of labeled glycerol replaced with unlabeled glycerol) as "$L$" = 100 − R




Complete hydrolysis and reutilization of labeled glycerol would result in labeled saturated triglyceride.




Hydrolysis to monoglycerides and resynthesis of triglycerides would produce no labeled trisaturated glyceride.

* = $C^{14}$ labeled. U = unsaturated acid. S = saturated acid.

**Fig. 5.** Rational for determination of the degree of hydrolysis and of the glyceride species absorbed.

To test which possibility had occurred the labeled fat was mixed with saturated triglycerides and fed. The rationale for this study is diagramed in Fig. 5. In this example labeled unsaturated and unlabeled saturated triglycerides were administered in equal proportions: If all the triglycerides were completely hydrolyzed, and the labeled glycerol reutilized, some labeled trisaturated triglyceride would have been formed. If, however, the two compounds were hydrolyzed only as far as the monoglycerides, no labeled saturated triglyceride could be formed. Similarly, the theoretical relative specific activities of the various glyceride fractions can be calculated for any degree of hydrolysis. These calculations were made and compared to the experimental results (Table 2). It may be seen that the experimental results fit only the conditions that no

**Table 2.** CALCULATED RELATIVE SPECIFIC ACTIVITIES OF THE GLYCEROL OF HYPOTHETICALLY POSSIBLE ACETONE-ALCOHOL SOLUBLE AND INSOLUBLE LYMPH GLYCERIDES AFTER THE INGESTION OF EQUAL PARTS OF GLYCEROL-LABELED CONJUGATED TRILINOLEIN AND UNLABELED SATURATED TRIGLYCERIDE

| | *Complete hydrolysis*[a] | *No hydrolysis* | *Hydrolysis to diglycerides* | *Hydrolysis to monoglycerides* | *Found* |
|---|---|---|---|---|---|
| Acetone-soluble | 40 | 80 | 75 | 60 | 60 |
| Acetone-insoluble | 100 | 0 | 0 | 60 | 59 |

[a] Reutilization of labeled glycerol.

completely hydrolyzed glycerol was reutilized and that only mono- but no di- or tri-glycerides were absorbed.

Confirmation that hydrolyzed glycerol is not reutilized has come from Wagner and Ritzel (18). These authors fed deuterium labeled glycerol, free and as triacetin, and found only traces in the lymph lipides. In the same report they presented data to show that triolein, deuterium labeled in the glycerol fraction, is only 24–53% hydrolyzed before its appearance in lymph fat, a figure close to that obtained in our laboratory.

That free glycerol, fed as such, or as glycerol phosphate, does not participate in glyceride resynthesis has been amply confirmed (18, 19). Furthermore, Borgström has been unable to incorporate glycerol and and fatty acids with pancreatic lipase in vitro under physiological conditions (11).

Since free glycerol or glycerol phosphate are not the precursor of glyceride glycerol, what is its precursor? The most logical compounds are glyceraldehyde or dihydroxyacetone. At the time this question arose, we had in our laboratory a few milligrams of 1-palmitoxy-3-hydroxyacetone labeled in the ketone and palmitic acid fractions. This compound was an intermediate in the synthesis of our labeled glycerol. (20) The material was fed to rats and appeared in the lymph as triglyceride, with the specific activity almost unchanged, acting very similar to labeled monopalmitin.

The appearance of labeled triglycerides in the lymph after the ingestion of either palmitoxy-hydroxyacetone or monopalmitin, with no loss of specific activity, indicates that just enough hydrolysis had occurred to permit the triglyceride synthesis. This suggested an enzymatically controlled reaction, such as would take place intracellularly, rather than digestion in the lumen of the intestine. That is, it seemed reasonable that monoglycerides are absorbed unchanged and converted to triglycerides in the intestinal mucosa.

A corollary of this hypothesis would be that phospholipides are not

Table 3. Relative Specific Activities of the Glycerol of Mucosa and Lymph Triglycerides and Phospholipides after the Ingestion of Glycerol-labeled Triglycerides

| *Mucosa* | | *Lymph* | |
|---|---|---|---|
| *Triglycerides (%)* | *Phospholipides (%)* | *Triglycerides (%)* | *Phospholipides (%)* |
| −154[a] | 38–42 | 54–73 | 46 and 48 |

[a] This factor may be explained if the mucosa glycerides contained appreciable quantities of unlabeled fatty acids.

intermediate in the resynthesis of triglycerides. Studies have been made in our laboratory to test this hypothesis by comparing the specific activity of ingested triglyceride, mucosa triglyceride and phospholipide, and lymph triglyceride and phospholipide (Table 3). The relative specific activities $R^3$, of the lymph and mucosa phospholipide glycerol were between 38 per cent and 48 per cent, while those of the triglycerides were between 54 per cent and 73 per cent. It thus seems clear that phospholipide must be formed from triglycerides rather than the reverse.

Gidez and Karnovsky have just informed me that they have unpublished data also demonstrating that the mucosa phospholipide glycerol has a lower relative specific activity than the triglycerides. A surprising observation was the presence in the lumen of the intestine of phospholipide glycerol with a relative specific activity of 100.

The demonstrated limited capacity of lipase to hydrolyze monoglycerides, as well as their rapid absorption, the proven fact that hydrolyzed glycerol does not participate in glyceride resynthesis, and the relative specific activities of labeled glycerol in ingested and lymph triglycerides and in mucosa and lymph phospholipide, have tempted me to construct a modified hypothesis of fat absorption (Fig. 6). Considering, first, the changes when monoglycerides alone are fed, the hypothesis that these are absorbed into the mucosa and there converted to triglycerides with no loss of glyceride glycerol, fits the experimental data. Under these conditions there would be no loss in relative specific activity.

The hypothesis for the mechanism of the digestion and absorption of triglycerides is diagrammatically presented in Fig. 7.

Triglycerides are hydrolized in the intestine to monoglycerides. The absorbed fatty acids combine in the mucosa with the absorbed monoglycerides and with the glycerol precursor in the proper proportions to produce triglycerides with the observed relative specific activity of 60 per cent. This step requires the postulate that monoglycerides and endogenous glycerol (or its precursor) compete for the absorbed fatty acids in the ratio of 3:2. Two molecules of this triglyceride may then combine

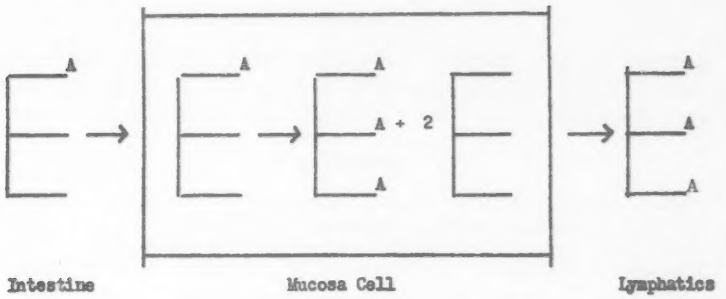



Fig. 6. Absorption of monoglycerides.

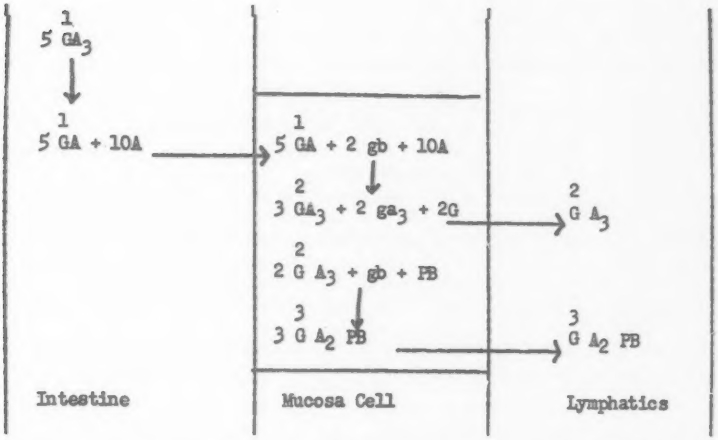



Fig. 7. Absorption of triglycerides. *G*, Labeled glycerol; *gb*, unlabeled glycerol precursor. *1*, Relative specific activity = 100; *2*, relative specific activity = 60; *3*, relative specific activity = 40.

with one molecule of glycerol precursor and one molecule of a phosphorylated base to produce phospholipide whose glycerol, therefore, has a relative specific activity of 40 per cent.

This scheme is presented as a working hypothesis only. Actually, there may be up to 10 per cent hydrolyses of monoglycerides in the intestine. Also, a number of alternative schemes could be designed which would fit the same data. However, this scheme seemed to be the simplest and most reasonable. I hope it may be of some help in clearing up the many mysteries which remain unanswered in this intriguing problem.

## SUMMARY

A review of the mechanism of digestion and absorption of fat has been presented. A new working hypothesis is suggested, based on the uses of tracer techniques.

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# Reproducibility and Validity of Sodium Sulfate Fractionation of Proteins in Plasma and Knee Joint Fluid

*O. H. Gaebler, W. T. Beher, J. W. Sigler, and R. R. Galpin*

Simple and rapid methods of determining plasma proteins, which yield results comparing favorably with those obtained by electrophoresis, have been sought by several investigators. The procedure of Milne seemed of particular interest to us, since it differs from the widely used one of Howe only with respect to the concentrations of sodium sulfate employed. The dual purpose of the present study was, first, to secure an additional series of normal values for serum proteins determined by the method of Milne, and second, to ascertain whether his reported agreement between salt fractionation and electrophoretic separation of serum applies to knee joint fluid. A component moving more rapidly than albumin was noted in all knee joint fluids examined, and was studied further by means of electrophoresis-convection (1).

## MATERIALS AND METHODS

Blood samples were drawn from the cubital vein, during mid-morning, from 16 apparently normal staff members who were seated during venepuncture. About an hour after clotting, serum was separated by centrifugation. Analyses were made in duplicate or triplicate by the method of Milne.

Knee joint fluids were brought to the laboratory shortly after aspirations had been performed in the Arthritis Clinic of the Hospital. Veronal buffer, *p*H 8.6 and ionic strength 0.1, was used in electrophoretic studies.

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We are indebted to Dr. James C. Mathies for carrying out the electrophoresis-convection experiment; to Mr. R. B. Scott and Mr. Ernest J. Schoeb of the Research Laboratories, Parke, Davis and Company, for electrophoretic analyses; and to the Michigan Chapter, Arthritis and Rheumatism Foundation, for a grant in support of this study.

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Each specimen was diluted 1:1 with this buffer and dialyzed against it for 16 to 24 hours before being run. The Aminco apparatus was used, and duration of electrophoresis was 120 minutes. Electrophoresis-convection had previously been applied, in this laboratory, to purification of alkaline phosphatase by Mathies, who carried out the experiment reported in this paper.

## RESULTS

In Table 1 are presented the data on serum. The finding of Milne that there is no difference between results for males and females was confirmed. Our normal values also coincide with his, and, as already stated, represent values in persons who were seated for a few minutes in the course of their regular duties. Although Rowe in 1916 recognized that exercise and posture alter plasma protein values, and Wolfson and Cohn have quite recently stated that the change from a standing to a recumbent position

**Table 1.** VALUES FOR FIBRINOGEN, BY THE METHOD OF WU, AND FOR SERUM PROTEINS BY THE SODIUM SULFATE FRACTIONATION PROCEDURE OF MILNE, IN NORMAL ADULT MALE AND FEMALE HUMAN SUBJECTS

| *Subject* | *Sex* | *Fibrinogen* (Gm. %) | *Serum proteins* (Gm. %) | | | | *Serum proteins* (% total serum protein) | | | *Serum N.P.N.* (mg. %) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | *Total* | *Euglobulin* | *Pseudoglobulin* | *Albumin* | *Euglobulin* | *Pseudoglobulin* | *Albumin* | |
| O. H. G. | M | | 7.22 | 1.76 | 0.86 | 4.60 | 24.4 | 11.9 | 63.7 | 32.6 |
| M. J. S. | M | 0.27 | 7.56 | 1.56 | 1.10 | 4.90 | 20.6 | 14.6 | 64.8 | 29.8 |
| W. T. B. | M | 0.31 | 7.60 | 1.81 | 1.07 | 4.72 | 23.8 | 14.1 | 62.1 | 29.6 |
| R. S. | M | | 7.78 | 1.78 | 1.30 | 4.70 | 22.9 | 16.7 | 60.4 | 28.0 |
| W. T. H. | M | | 7.88 | 2.06 | 1.26 | 4.56 | 26.2 | 16.0 | 57.8 | 27.8 |
| P. D. B. | M | 0.33 | 7.70 | 1.69 | 1.16 | 4.85 | 21.9 | 15.1 | 63.0 | 33.8 |
| J. C. M. | M | 0.36 | 7.40 | 1.86 | 0.92 | 4.62 | 25.1 | 12.4 | 62.5 | 26.8 |
| H. H. S. | M | 0.25 | 7.77 | 1.61 | 1.58 | 4.58 | 20.7 | 20.3 | 59.0 | 31.6 |
| F. J. O. | M | 0.23 | 6.91 | 1.81 | 0.92 | 4.18 | 26.2 | 13.3 | 60.5 | 28.9 |
| E. H. W. | F | 0.26 | 7.75 | 2.44 | 1.25 | 4.06 | 31.5 | 16.2 | 52.3 | 20.8 |
| E. M. C. | F | 0.28 | 7.46 | 1.51 | 1.42 | 4.53 | 20.2 | 19.0 | 60.8 | 24.2 |
| B. M. | F | 0.29 | 7.85 | 2.18 | 0.85 | 4.82 | 27.8 | 10.8 | 61.4 | 30.2 |
| E. G. | F | 0.31 | 7.16 | 2.04 | 0.97 | 4.15 | 28.5 | 13.5 | 58.0 | 25.4 |
| B. C. | F | 0.32 | 7.47 | 1.91 | 0.69 | 4.87 | 25.6 | 9.2 | 65.2 | 23.6 |
| Mean, males | | 0.29 | 7.54 | 1.77[a] | 1.13 | 4.63 | .. | .. | .. | .. |
| Mean, females | | 0.29 | 7.54 | 2.02[a] | 1.04 | 4.49 | .. | .. | .. | .. |
| Mean, all subjects | | 0.29 | 7.54 | 1.86 | 1.10 | 4.58 | 24.7 | 14.5 | 60.8 | 28.1 |
| Standard deviation | | ±0.04 | ±0.29 | ±0.25 | ±0.25 | ±0.27 | ±3.3 | ±3.0 | ±3.35 | .. |

[a] Significance of difference in means, $t = 1.94$; $p > 0.05, < 0.1$.

Table 2. Analyses of Knee Joint Fluids by Sodium Sulfate Fractionation and Electrophoresis

| Sample number | Albumin (Gm.%) | | Pseudoglobulin by sodium sulfate (Gm.%) | $\alpha_1$ plus $\alpha_2$ globulins by electrophoresis (Gm.%) | Euglobulin by sodium sulfate (Gm.%) | $\beta$ plus $\gamma$ globulins by electrophoresis (Gm.%) |
|---|---|---|---|---|---|---|
| | Sodium sulfate | Electrophoresis | | | | |
| 1-L[a] | 1.85 | 1.78 | 0.79 | 0.56 | 2.19 | 2.49 |
| 2-R[a] | 1.98 | 2.00 | 0.70 | 0.70 | 2.12 | 2.12 |
| 3-L | 1.40 | 1.91 | 1.52 | 0.55 | 1.95 | 2.41 |
| 4-R | 1.76 | 2.14 | 1.04 | 0.62 | 2.07 | 2.10 |
| 5-L | 1.35 | 1.99 | 1.14 | 0.52 | 2.06 | 2.04 |
| 6-R | 1.64 | 1.89 | 0.87 | 0.70 | 2.15 | 2.08 |
| 7-L | 1.18 | 1.92 | 1.44 | 0.57 | 2.08 | 2.20 |
| 8-R | 1.65 | 2.00 | 1.17 | 0.63 | 2.02 | 2.18 |

[a] L left knee; R right knee.

has hydrostatic effects which may alter serum protein values 15 per cent, this information is still received with skepticism at times. In Table 1 fibrinogen values, determined by the method of Wu, are included for completeness. Specimens for this purpose were oxalated with 2 mg. of potassium oxalate per ml.

In Table 2 are presented the values for proteins in knee joint fluid. Actually, we analyzed 18 knee joint fluids by the method of Milne, but only 8, in which comparisons between the electrophoretic and salt fractionation results were made, are included in the table. These were all obtained at different visits from the same patient. In Samples 3 to 8, albumin values obtained by the sodium sulfate method were well below the electrophoretic ones, and pseudoglobulin considerably exceeded the sum of the $\alpha_1$ and $\alpha_2$ electrophoretic fractions. This suggests entrainment of albumin, when the high concentration of sodium sulfate required for precipitation of pseudoglobulin is used in the analysis of knee joint fluid. Whatman #50 filter paper was used throughout, since it has been reported (2) that this minimizes errors due to adsorption of albumin on filter paper. Results for euglobulin compared fairly closely with the sum of $\beta$ and $\gamma$ fractions, in six of the eight samples.

Results of electrophoresis-convection were simple. Pooled knee joint fluids from 4 patients were centrifugalized for 30 minutes at 5° and 3300 RPM to remove a flocculent precipitate. A 10 ml. sample (Sample 1) of the supernatant was diluted with an equal volume of phosphate buffer, *p*H 7.2 and 0.1 ionic strength, and dialyzed against four 4 liter portions of this buffer. The supernatant was still faintly turbid, and the solution was viscous. Electrophoresis-convection was carried out for 6 hours at

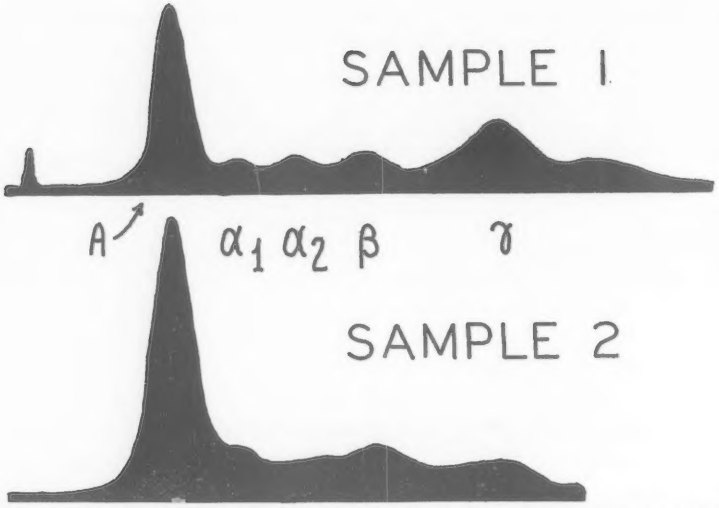


**Fig. 1.** Electrophoretic patterns (ascending boundaries) of pooled knee joint fluids (Sample 1) and fraction thereof (Sample 2) taken from lower chamber of electrophoresis-convection apparatus.

0.5 amp., the potential drop across the apparatus being 12 v. The bottom fraction was then sampled (Sample 2). The electrophoretic patterns before and after electrophoresis-convection are shown in Fig. 1. The fast moving component was not concentrated in the bottom fraction, as one had reason to expect, but disappeared. Adherence to the membranes of the apparatus is one of several possible explanations. In any case, use of this procedure to concentrate the component for study does not appear feasible. In the 8 samples, this component amounted to only 0.04–0.10 per cent of the total protein.

## SUMMARY

Blood was drawn from 14 laboratory workers under conditions similar to those under which blood samples are taken from ambulatory clinic patients. The sera were analyzed for the various proteins by the method of Milne. Eighteen knee joint fluids were analyzed by the same method; and 8 fluids from one patient were also analyzed electrophoretically. Results for these 8 fluids, and for all of the sera, are presented in tabular form and discussed. A component moving faster than albumin was noted in all knee joint fluids. This could not be concentrated from a

pooled lot from 4 patients by electrophoresis-convection, but disappeared instead.

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# Colorimetric Determination of Blood Arginase

*Max M. Friedman and Edythe Becker*

An extensive literature on arginase activity in tissues and tissue homogenates has accumulated in recent years. Roberts (1) has studied the arginase activity of liver, kidney, and epidermis of the Swiss mouse and of a transplantable squamous cell carcinoma. He found that liver had the greatest activity, followed by carcinoma, epidermis, and kidney. Sano (2) isolated an enzyme from rabbit intestinal mucosa which formed urea at *p*H 9.2 from D(−)arginine, but not from natural L(+)arginine. He called this enzyme "heteroarginase." Dounce *et al.* (3) found high concentrations of arginase in isolated nuclei from rat liver. Van Scott (4) found that normal skin (female breast) contained considerable arginase activity, predominantly in the epidermis. Liener and Schultze (5) found in acute uremia of newborn rats a significant increase of liver arginase activity. These workers also found a high degree of correlation between the arginase activity of the liver and the urea level of the blood.

Less attention has been directed towards the arginase activity of blood. Covolo and West (6), in a study of the arginase activity of red blood cells, found this activity quite constant in a given individual and slightly higher in women than in men. Of the various diseases studied, only in infectious mononucleosis was there found to be any increase in red blood cell arginase. Kochakian *et al.* (7) found the arginase activity of red blood cells higher in males than in females, and that the values for given individuals varied considerably from time to time. Their results were not in accord with the findings of the previous investigators (6). They also found much variation in pathologic conditions.

The presence of arginase has been reported in the red blood cells, but not in the plasma, of human blood (8). We attempted various procedures for hemolysis of the red blood cells in an effort to determine the blood

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arginase activity in patients with carcinoma. Only negligible enzyme activity was obtained with blood not previously hemolyzed, and this activity was increased by the addition of increasing amounts of distilled water to the blood. As will subsequently be shown, the maximum activity was obtained only with the use of saponin, upon which principle this method is based. The procedure adopted was similar to the colorimetric method of Van Slyke and Archibald (9, 10) with some modifications in its application to blood arginase. The enzyme is permitted to act on a buffered arginine substrate and the liberated urea is then measured colorimetrically. Since no enzyme other than arginase is present in the blood which is known to yield urea from arginine (9), we have adopted as *a unit of blood arginase that amount of activity which under the conditions of the described procedure will yield 1 micromole of urea (0.06 mg.) per milliliter of blood.*

## REAGENTS

*Manganese chloride* ($MnCl_2 \cdot 4H_2O$), 20.0% solution.

*Arginine substrate*, to 9.0 gm. of L-arginine monohydrochloride (Merck), add 3.2 ml. of 9.0N NaOH ($CO_2$ free) and make to 50 ml. with water. Solution is 0.85M and *p*H 9.5.

*Trichloroacetic acid*, 15% solution.

*Sulfuric-phosphoric acid mixture*, 90 ml. conc. $H_2SO_4$ and 270 ml. 85% phosphoric acid are poured into water and made to 1000 ml.

*Saponin*, 0.05% solution.

*α-isonitrosopropiophenone* (*Eastman Kodak*), 3.0% solution in 95% ethanol.

*Urea, stock*, 67.3 mg. urea dissolved in 100 ml. water.

*Urea, working standard*, dilute urea stock solution by adding 1 part stock to 24 parts sulfuric-phosphoric acid mixture.

*Arginine-trichloroacetic acid mixture*, to 5.0 ml. of arginine substrate add 10.0 ml. of trichloroacetic acid and 5.0 ml. water.

## PROCEDURE

### Hemolysis

To 1.0 ml. of oxalated blood add 9.0 ml. saponin solution, mix well, and let stand 30 minutes at room temperature.

### Activation

Add 0.5 ml. of 20% manganese chloride to the above solution and let stand 2 hours at room temperature. This is necessary for arginase activation by $Mn^{++}$. Two aliquots each of 2.0 ml. are transferred to test tubes

marked $T_1$ (total urea) and $T_2$ (preformed urea). To $T_1$ add 1.0 ml. arginine substrate and incubate at 25° for exactly 10 minutes, followed immediately by 2.0 ml. of 15% trichloroacetic acid. To $T_2$ add 2.0 ml. trichloroacetic acid (to prevent enzyme activity), followed by 1.0 ml. arginine substrate. Shake both tubes, let stand 15 minutes, and filter. A reagent blank is prepared simultaneously by adding 0.1 ml. manganese chloride to 1.9 ml. saponin, 2.0 ml. trichloroacetic acid, and 1.0 ml. arginine substrate, shaking, and filtering. One blank may serve for any one series of determinations.

### Urea Determination

To 2.0 ml. of the above filtrates ($T_1$, $T_2$, and blank) add 15.0 ml. sulfuric-phosphoric acid mixture to each, mix, and transfer 10.0 ml. portions into pyrex test tubes. To each add 0.5 ml. $\alpha$-isonitrosopropiophenone, mix, and place in a boiling water bath for one hour in the dark. Test tubes should be fitted with a one-hole rubber stopper through which is passed a short heavy-walled capillary tube of 0.5–1.0 mm. bore. After removing from the boiling water bath place the tubes in a bath of 50–55°, transfer into warm curvettes, and read immediately in the colorimeter with a 540 m$\mu$ filter, the reagent blank set at 100 per cent transmission.

### Preparation of Urea Curve

To 1, 2, 3, 4, 5, and 6 ml. of working urea standards as prepared under *reagents* are added respectively 8, 7, 6, 5, 4, and 3 ml. sulfuric-phosphoric acid mixture (total volume 9.0 ml.). To each tube is added 1.0 ml. arginine-trichloroacetic acid mixture and the urea is determined as described under *procedure*, with the addition of 0.5 ml. $\alpha$-isonitrosopropiophenone, heat, etc. The blank consists of 9.0 ml. sulfuric-phosphoric acid mixture and 1.0 ml. arginine-trichloroacetic acid mixture, and is treated in the same manner as the standards.

### Calculation

The urea working standards are so prepared that 1.0 ml. is equivalent to 10 blood arginase units as calculated from the aliquots of this procedure. A plot of the standard urea curve using the Leitz colorimeter and a 535 m$\mu$ filter is shown in Fig. 1. For subsequent determinations of blood arginase it is only necessary to subtract $T_2$ from $T_1$ and obtain the results directly in blood arginase units.

### Discussion

The method described for blood arginase is based on the formation of urea by action of the enzyme on a buffered solution of arginine. After

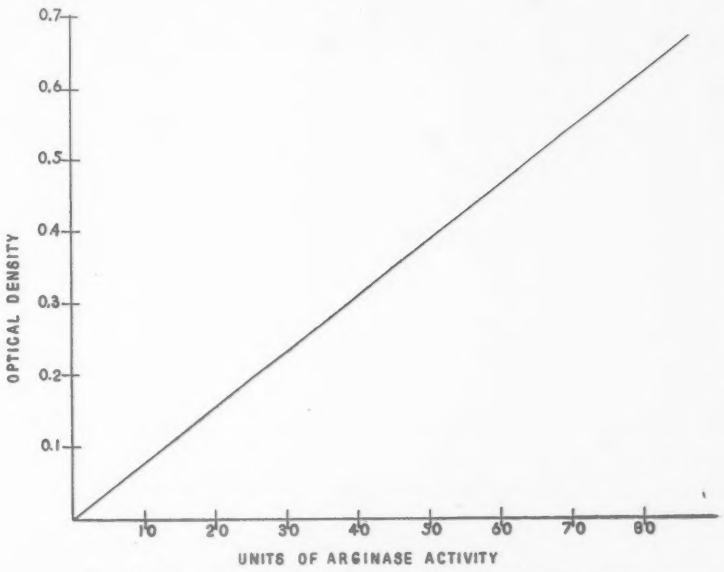


**Fig. 1.** Plot of standard urea curve in terms of arginase activity.

hemolysis of the red blood cells, the enzyme is activated for two hours at room temperature in the presence of 0.5 ml. of 20% manganese chloride. The conditions for incubation are those of Van Slyke and Archibald (9) using an arginine substrate of 0.85M at a *p*H of 9.5. For incubation 1 part of substrate is added to 2 parts of test solution giving a final arginine concentration of 0.28M. The incubation is allowed to proceed for 10 minutes at 25°. With this concentration of buffer it is not necessary to prepare a standard reference curve (9) since the urea formed is directly proportional to the amount of arginase activity. To further test this assumption a control experiment was performed using lyophilized beef liver arginase[1]. An aqueous solution of 1.0 mg. arginase per ml. was prepared, and diluted 1:20 with saponin. One milliliter of this solution now contained 0.05 mg. of lyophilized enzyme. To 1.0 ml. serum (arginase-free) was added 1, 2, 3, and 4 ml. of this diluted enzyme, made to 10 ml. with saponin, and the activity determined as described under *procedure*. The results are plotted in Fig. 2 and show a linear activity-concentration curve.

Since arginase is not present in human serum, but only in the red blood

[1] Worthington Biochemical Corporation, Freehold, New Jersey.

Table 1. Blood Arginase Activity as a Function of Time of Hemolysis with Water and Saponin

| *Sample No.* | *Hemolysis* (min.) | *Water* (units) | *Saponin* (units) |
|---|---|---|---|
| 1 | 15 | 17.0 | 33.2 |
| | 30 | 19.8 | 42.5 |
| | 180 | 18.0 | 39.0 |
| 2 | 30 | 25.3 | 53.5 |
| | 180 | 17.8 | |
| 3 | 30 | 14.3 | 36.0 |
| 4 | 30 | 24.0 | 56.8 |
| 5 | 30 | 24.0 | 63.2 |
| 6 | 15 | | 48.0 |
| | 30 | | 54.7 |
| 7 | 30 | | 52.3 |
| | 60 | | 52.0 |

cell, the procedure for hemolysis was investigated. Previous investigators (6, 7) hemolyzed the red blood cell with water and determined the arginase activity of the suspension. To expedite this step we attempted to use a lysing agent such as saponin and consequently found a marked increase in activity when compared to water alone. A series of determinations was run using 1 part of blood to 9 parts of water and 1 part of blood to 9 parts of saponin, and these were permitted to stand for varying periods of time for hemolysis. The results of these analyses are shown in Table 1. It is obvious from these data that with the use of saponin much larger amounts of arginase activity are obtained. The optimum enzyme activity was found with a 1:10 saponin dilution after 30 minutes standing, as adopted in this procedure. Rossiter (11) has also demonstrated that surface-active substances such as saponin liberate large amounts of active phosphatase from leukocytes in suspension, and Valentine and Beck (12) used saponin for the same purpose in the determination of acid and alkaline phosphatases of leukocyte suspensions.

Trichloroacetic acid was substituted for metaphosphoric acid (9) since it was found that the latter produced turbid supernatants on centrifugation or filtration.

As previously found (8), we could demonstrate no arginase activity in human sera. There was only negligible activity in freshly drawn blood determined without preliminary hemolysis.

For precise results it is also necessary to determine the preformed urea, since the activity is measured by the difference in urea before and after incubation. However, since the urea after incubation averages about ten times that of the preformed urea, it would be possible in the absence of

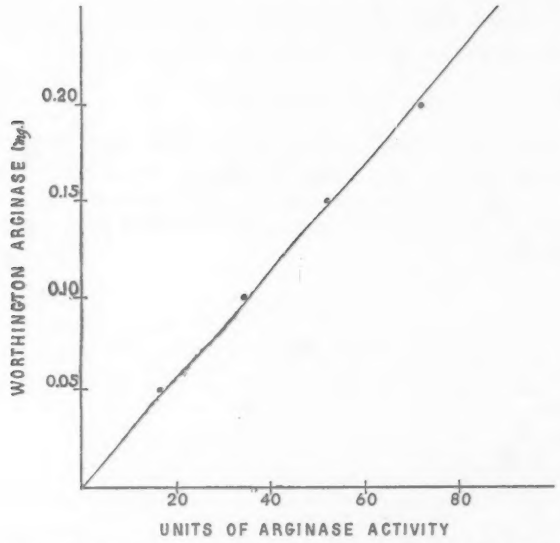


**Fig. 2.** Linear activity-concentration curve using lyophilized beef liver arginase.

**Table 2.** DISTRIBUTION OF BLOOD ARGINASE VALUES IN NORMAL ADULTS

| *Arginase activity* (Units) | *Number of subjects* |
|---|---|
| Below 30 | 0 |
| 30–40 | 18 |
| 41–50 | 33 |
| 51–60 | 29 |
| 61–70 | 11 |
| 71–80 | 8 |
| Above 80 | 1 |

uremia to use an average value for urea as the control figure ($T_2$) with only a small error and thus simplify the procedure for clinical purposes. Also the preformed urea by any other method would be suitable.

## RESULTS

A series of determinations was carried out on normal adults, which included 12 laboratory workers and 88 Red Cross blood donors. The distribution of results are shown in Table 2. The average value was found to be 51.7 units with a standard deviation of $\pm 12.4$ units, and a range of

30.1 to 83.1 units. Most of the normal subjects were males and no attempt at this time was made to study sex differences.

## SUMMARY

A colorimetric procedure for blood arginase has been described, based on hemolyzing the red blood cells with saponin and determining the urea formed by action of the enzyme on an arginine substrate.

The range of blood arginase in a series of normal human subjects has been presented.

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# Purification of Chromium (VI) for Use in the PBI Determination

*W. Burkett Mason and Santo Coco*

In connection with the determination of serum protein-bound iodine (PBI), it is occasionally advantageous to have available reagents with a very low iodine blank. Of the reagents required in a PBI procedure employing dialysis, wet combustion with $H_2SO_4$ and Cr (VI), volatilization of iodine after liberation with $H_3PO_3$, and estimation of iodine by means of the Ce (IV)–As (III) reaction, the Cr (VI) contributes most of the iodine blank.

Although $CrO_3$ and $Na_2Cr_2O_7$ can be obtained having as little as $5 \times 10^{-9}$ Gm. I per Gm. Cr, the value is often much higher. Of various procedures undertaken for preparing Cr (VI) which is essentially free of iodine, conversion to chromyl chloride has proved most satisfactory. Using the method recommended by Fernelius (1), a batch of heavily contaminated $CrO_3$ containing $88 \times 10^{-9}$ Gm. I per Gm. Cr, for example, was converted to crude $CrO_2Cl_2$ containing $1 \times 10^{-9}$ gm. I per Gm. Cr, with a yield of 66 per cent. Studies utilizing $I^{131}$ (as NaI) added to the initial $CrO_3$ solution confirmed the efficiency of the separation by indicating better than fiftyfold purification.

The crude chromyl chloride can be diluted with water and used with concentrated sulfuric acid for wet combustion of serum in the PBI determination. The digestion, however, had best be carried out in a well-ventilated hood because of the noxious fumes evolved.

## SUMMARY

Chromium (VI) which is essentially free of iodine can be prepared by converting $CrO_3$ to $CrO_2Cl_2$. The crude chromyl chloride can be used with concentrated sulfuric acid for wet combustion of serum in the PBI determination.

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# Iron Content of Tissues in Exogenous and Endogenous Hemochromatosis

*Bernard Klein, Benjamin S. Gordon, and Irving Graef*

Hemochromatosis is a disease marked by the pathologic appearance of large amounts of iron-containing pigment, hemosiderin, in various tissue cells, and is associated with periportal cirrhosis of the liver and pancreatic fibrosis. Three types of hemochromatosis have been recognized:

1. Idiopathic or endogenous hemochromatosis, wherein the intestinal "mucosal block" preventing excessive absorption of iron from the intestine is ineffective, and allows a gradual but persistent accumulation of surplus iron (1).
2. Exogenous hemochromatosis, in which multiple blood transfusions are said to be the underlying cause of excess iron accumulation (2).
3. Dietary hemochromatosis (3), in which extrinsic dietary factors are believed to be responsible for the breakdown of the mucosal block's regulation of iron absorption.

We have had the opportunity of studying several cases of the exogenous, or transfusion, type of hemochromatosis and analyzing the tissues at postmortem for iron. The results were compared with values obtained from the endogenous type for the purpose of differentiating chemically rather than histologically the two dominant forms of the disease. The pathologic and histologic aspects have been reported earlier (4). Cases of the third type were not available to us.

## METHODS

A history of anemia of long duration was found in all exogenous cases studied. Multiple blood transfusions and hematinic therapy over a period of 1 to 8 years had been used unsuccessfully.

For comparison, the organs of several patients with long-standing

From the Laboratory and Medical Services, Veterans Administration Hospital, Bronx, N. Y.

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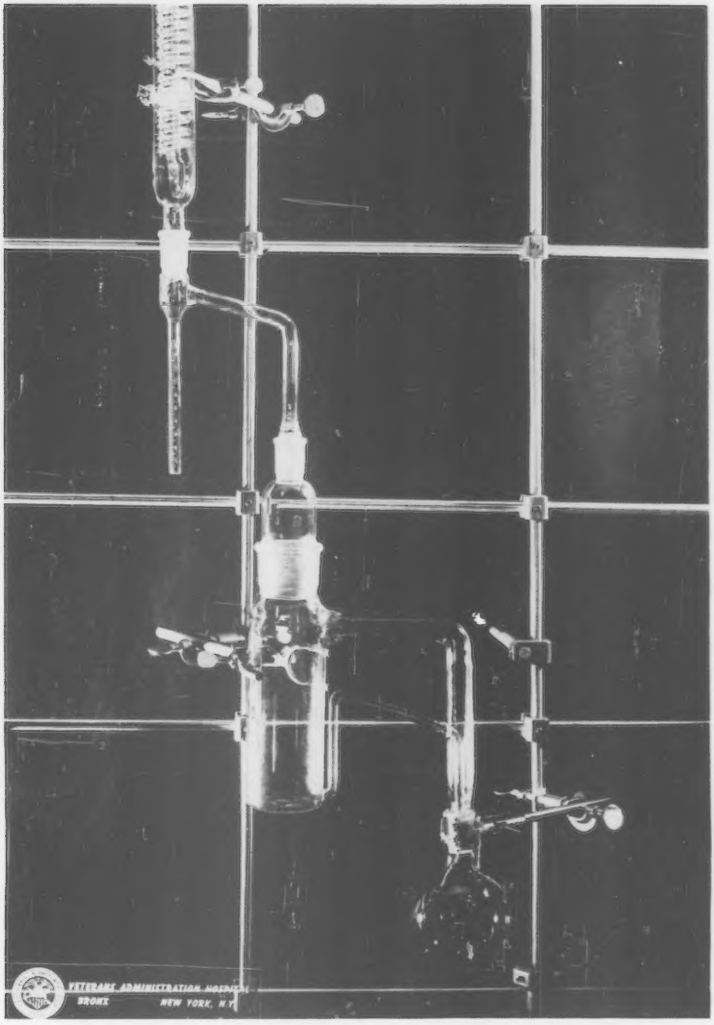


**Fig. 1.** Apparatus used for simultaneous drying and fat extraction of tissues by azeotropic distillation. The upper joint of the extraction chamber is 55/50; all other joints are 24/40.

anemia who had received multiple transfusions and who at autopsy did not reveal the anatomic features of hemochromatosis were analyzed for iron.

A representative 5–10 Gm. portion of tissue obtained at postmortem was washed free of blood with distilled water, blotted with cheesecloth, and placed in a 20 × 40 mm. thimble. Five to six such thimbles, comprising tissues from a single autopsy, were placed in the extraction chamber of the drying apparatus (Fig. 1) and subjected to simultaneous dehydration and extraction by azeotropic distillation with isopropyl ether. The distillation and extraction were continued for 1 hour after water was no longer given off after which the thimbles were removed to a vacuum desiccator and dried under reduced pressure for a half-hour after manometric pressure of the system had become constant. The contents of the thimbles were pulverized and stored in a desiccator.

The samples were analyzed for iron by digesting 0.10 to 0.20 Gm. of dried tissue with 2 ml. 1:1 sulfuric-perchloric acid mixture. In some cases a few drops of 20% hydrogen peroxide was added terminally to clear the digest. The cooled digest was diluted to 50 ml. and aliquots of 1–5 ml. were taken for analysis by the Wong thiocyanate procedure (5).

## RESULTS

The iron content of tissues of 2 individuals without anemia or any metabolic iron disturbance, who had come to autopsy is given in Table 1. These serve as control values and are in keeping with the values obtained by Ramage and Sheldon (9) in their series of analyses of normal tissues.

Table 2 shows the iron content of tissues in 2 cases of endogenous hemochromatosis. Markedly increased amounts of iron are deposited and are the distinct chemical manifestation of the pathologic process.

Table 2 shows also the comparable values obtained in the analysis of tissues in 5 cases of exogenous (transfusion) hemochromatosis. Again the picture is that of excessive iron deposition with values in the same magnitude as the tissues analyzed by Ramage and Sheldon, but somewhat greater than the values obtained in our cases of the endogenous form. The tissue-iron values obtained in the series of anemia cases

**Table 1.** IRON CONTENT OF NORMAL TISSUE

| *A #* | *Liver* | *Heart* | *Spleen* | *Pancreas* |
|---|---|---|---|---|
| 2303 | 1.61[a] | 0.38 | 3.04 | 0.49 |
| 2248 | 0.14 | 0.49 | 2.38 | 0.53 |

[a] All results are expressed as mg. Fe/Gm. dry-fat-free tissue.

Table 2. Iron Content of Tissues in Hemochromatosis and Anemia

| *A #* | *Kidney* | *Liver* | *Lung* | *Heart* | *Spleen* | *Pancreas* |
|---|---|---|---|---|---|---|
| Endogenous Hemochromatosis | | | | | | |
| B39119 | 2.62[a] | 4.88 | 4.77 | 0.74 | .. | 10.4 |
| B37926 | 1.49 | 12.6 | 5.8 | 0.84 | .. | 12.5 |
| Exogenous Hemochromatosis | | | | | | |
| 1115 | 4.68 | 23.7 | 2.63 | 2.78 | 7.11 | 18.4 |
| 1332 | 3.33 | 22.9 | 3.30 | 2.99 | 3.30 | 15.2 |
| 1032 | 4.92 | 41.2 | 4.08 | 6.77 | .. | 21.0 |
| 1383 | 2.41 | 39.7 | 5.21 | 0.29 | 3.39 | 2.57 |
| H | 0.005 | 9.02 | 4.21 | 3.94 | .. | 4.18 |
| Refractory Anemias with Hemosiderosis | | | | | | |
| 1597 | 1.97 | 21.0 | 3.44 | 1.23 | 6.84 | 9.21 |
| 1598 | 3.43 | 32.3 | .. | 0.29 | .. | .. |
| 480 | .. | .. | 4.18 | .. | .. | 8.35 |
| 1608 | 4.11 | 9.32 | 4.36 | 1.55 | 21.3 | 6.0 |

[a] All results are expressed as mg. Fe/Gm. dry fat-free tissue.

without fibrosis overlap, and are almost indistinguishable from the values obtained in the two true hemochromatotic types.

On the basis of results obtained, chemical analysis of tissues for iron does not decisively differentiate between the two varieties of the disease. There is considerable overlap.

## DISCUSSION

### Method

There are some points to be noted in the preparation of the samples for analysis. When dried by the customary process of oven-drying to constant weight, the tissues turned dark and hardened to a resin-like mass containing varying amounts of fat. This resulted in failure to get reproducible results on duplicate analyses of the same tissue, although in all other respects our technic when applied to blood and standard recovery studies was acceptable (average: 97.3 per cent). An improvement in the drying process was necessary. To this end, the drying-extraction apparatus of Kaye, Leibner, and Connor (6) was redesigned to permit the simultaneous dehydration and fat extraction by azeotropic distillation of representative portions of the organs from an entire autopsy in a single operation. After this treatment the organ fragments were colorless and easily powdered. Handling and weighing were simplified. The samples could be stored in a desiccator indefinitely. Duplicate analyses checked within 5 per cent.

Our data are therefore reported on a dry-fat-free basis and are in keeping both with the work of Pace and Rathbun (7), who showed that among laboratory animals the whole body fat varies from 1.5 to 35.8 per cent of body weight, and the plea of A. B. Hastings (8) that reports of tissue analysis be on a dry fat-free basis.

## Results

From the results obtained in our series of tissue analyses, it can be concluded that in terms of tissue iron the exogenous and endogenous types of hemochromatosis are indistinguishable. Nodular pigment cirrhosis, hepatomegaly, and fibrosis with extensive pigment deposition were found in both at autopsy. Intracellular pigment was found in various tissue cells and in macrophages. The histologic criteria for diagnosis were satisfied.

Chemical findings however, did not in all instances coincide with the histologic picture. For example, in Case H, more hemosiderin was observed histologically in the pancreas, which contained 4.18 mg. iron/Gm. of tissue, than in the pancreas of A 1165, which contained 18.4 mg./Gm. Similarly, in the spleen of A 1115, with 7.1 mg. iron/Gm., less hemosiderin could be demonstrated histologically than in the spleen of A 1383, which had 3.39 mg. iron/Gm.

On the other hand, the 4 patients with various anemias which had been treated with iron therapy and multiple transfusions showed extensive hemosiderin deposits as well as increased tissue iron. Pathologically, none of the fibrotic stigmata seen in hemochromatosis were in evidence here. Here, too, clear-cut differentiation by chemical analysis of tissue cannot be made. These observations may possibly cast some light on the order and sequence of the pathologic manifestations of the exogenous form of hemochromatosis. After multiple transfusions, the iron released by the eventual breakdown of red blood cells by normal metabolic processes or otherwise, is stored in tissues because no excretory mechanism exists (10). Excess amounts are then taken up by the parenchymous liver cells, spleen, and other organs and fibrosis of the liver and pancreas ensues.

## SUMMARY

1. Tissues from patients with the endogenous and exogenous (transfusion) forms of hemochromatosis were analyzed for iron content and their values compared.

2. Tissues from patients with long-standing anemia were analyzed for iron content.

3. The values obtained in all cases overlap and tissue-iron analysis cannot be used to differentiate the two forms of hemochromatosis.

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# Clinical Significance of the Magnesium : Calcium Ratio

## Technic for the Determination of Magnesium and Calcium in Biologic Fluids

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The Schwartzenbach titration of calcium and magnesium has been applied principally in the field of determination of total hardness in water (1–4). Due to its particular application in this field where many contaminants are possible, Diehl, Goetz, and Hach have published a table of the principal interfering substances, their maximum tolerated concentrations, and the various inhibiting reagents which may be added to the titration to eliminate this interference. These considerations, however, have been found superfluous when the method or a modification is applied to biologic fluids, since these substances (for example, chromium, manganese, and so on) are either absent or are present in very small quantities.

Titration with Versene[1] has been done by, among others, Greenblatt and Hartmann. These investigators used a solid indicator, ammonium purpurate, which like eriochrome Black T, the Schwartzenbach indicator, forms a colored complex with calcium but not with magnesium. In our study it was desirable to obtain values for both calcium and magnesium in serum. Therefore a method was developed which permitted the determination of both ions in serum.

### METHOD

#### Standards

Standards are prepared in concentrations comparable to those obtained in serum. All solutions are prepared from reagent-grade chemicals.

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[1] Trade name of ethylenediaminetetraäcetic acid; manufactured by Versenes, Inc., Framingham, Mass.

### Magnesium

Transfer exactly 50.0 mg. magnesium powder to a 1000-ml. volumetric flask. Add 600 ml. of distilled water and 50 ml. of 0.1N hydrochloric acid. Shake gently until the powder is dissolved. Wash down the walls of the flask with distilled water and dilute to the mark. Mix well by inversion. This solution contains 4.12 mEq./L. magnesium.

Since the magnesium standard is made up from calcium-free magnesium metal, the versene solution is standardized with this standard alone, in terms of mEq./L. divalent ion. Most commercial calcium salts contain as much as 0.5 per cent magnesium.

### Buffer Solution, pH 10 (4)

Mix 67.5 Gm. ammonium chloride and 570 ml. of concentrated ammonium hydroxide. Dilute to 1000 ml. with distilled water. Mix well and store in a tightly closed glass-stoppered bottle. Polyethylene ware may be used.

### Indicator Solution

Dissolve 0.5 Gm. eriochrome Black T (Eastman Organic Chemicals or Versenes, Inc.) in 100 ml. of distilled water. This solution is stable for several months.

### Stock Versene Solution

Dissolve 750 mg. of ethylenediaminetetraäcetic acid (or its equivalent of the di- or tetrasodium salt) in the minimum amount of 0.1N NaOH (about 80 ml. for the free acid, 40 ml. for the disodium salt, and distilled water for the tetrasodium salt) in a 1000-ml. volumetric flask, and dilute to the mark with distilled water. Mix well and store in a glass-stoppered borosilicate or polyethylene bottle. This solution is stable indefinitely.

### Standard Versene Solution

Dilute 100 ml. of the stock Versene solution to 1000 ml. in a volumetric flask with distilled water. Titrate against the magnesium standard and adjust with distilled water or stock Versene solution so that 1 ml. magnesium standard will require 8.0 $\pm$ 0.3 ml. One ml. of the standardized solution will then be equivalent to approximately 0.5 mEq. of divalent ion.

### Buffered Oxalate Solution (4)

To 35 Gm. ammonium chloride, add 1.5 Gm. ammonium oxalate and 3.5 ml. concentrated ammonium hydroxide. Dilute to 250 ml. with distilled water.

## Procedures

### Total Divalent Ions

Place 0.5 ml. of standard or unknown solution in a small Erlenmeyer flask. Add about 4 ml. of distilled water, 0.5 ml. buffer *p*H 10, and 2–4 drops of indicator solution. Titrate with standard Versene solution from a 5 ml. microburet (Koch type) until the solution turns from a pink to a pure blue color. This method is satisfactory with serum and spinal fluid.

### Magnesium

Place 1 ml. serum or spinal fluid in a 15-ml. centrifuge tube. Add 2.5 ml. distilled water and 0.5 ml. buffered oxalate solution. Mix well and let stand for 30 minutes at room temperature. Centrifuge at 2500 rpm for 10 minutes. Transfer 2 ml. of the supernatant fluid to an Erlenmeyer flask, add reagents, and titrate as for total divalent ions.

## Calculations

If, for example, Versene solution used was equivalent to 0.493 mEq./L. magnesium, this value becomes 0.986 mEq. per liter, as 0.5 ml. of sample is used. Thus 0.986 × ml. standard Versene solution = mEq./L. divalent or magnesium ions. The mEq./L. calcium ions is calculated by difference.

## RESULTS

An initial series of 10 normal sera was tested in duplicate by Versene titration and by the Denis (8) and permanganate (9) methods for magnesium and calcium, respectively. The results are shown in Table 1. The Versene determination of magnesium checked with the Denis method within 0.1 mEq./L., approximately the standard error of the mean. The calcium values by Versene titration varied from the permanganate values by as much as 0.48 mEq./L., approximately 24 times the standard error of the mean.

A similar series was carried out with 10 spinal fluids. The results are

**Table 1.** Correlation of Serum Calcium and Magnesium Values by the Versene Titration and the Permanganate and Ammonium Phosphate Methods

| Serum | Magnesium | | Calcium | | Versene/$NH_4HPO_4$ | Versene/$KMnO_4$ |
|---|---|---|---|---|---|---|
| | Versene (mEq./L.) | $NH_4HPO_4$ (mEq./L.) | Versene (mEq./L.) | $KMnO_4$ (mEq./L.) | | |
| 1. | 1.60 | 1.53 | 4.98 | 5.06 | 1.03 | 1.02 |
| | 1.61 | 1.59 | 4.97 | 5.08 | | |
| 2. | 1.35 | 1.31 | 5.60 | 5.62 | 1.01 | 1.00 |
| | 1.34 | 1.34 | 5.59 | 5.60 | | |
| 3. | 1.52 | 1.52 | 5.12 | 5.46 | 0.99 | 0.94 |
| | 1.52 | 1.55 | 5.13 | 5.40 | | |
| 4. | 1.00 | 1.09 | 4.46 | 4.40 | 0.93 | 1.01 |
| | 1.02 | 1.08 | 4.46 | 4.39 | | |
| 5. | 1.17 | 1.17 | 5.03 | 4.97 | 1.00 | 1.00 |
| | 1.18 | 1.17 | 5.01 | 5.02 | | |
| 6. | 1.89 | 1.87 | 4.36 | 4.84 | 1.00 | 0.91 |
| | 1.89 | 1.91 | 4.39 | 4.81 | | |
| 7. | 1.10 | 1.09 | 5.00 | 5.00 | 1.00 | 0.99 |
| | 1.08 | 1.09 | 5.00 | 5.04 | | |
| 8. | 1.35 | 1.27 | 5.51 | 5.58 | 0.97 | 0.98 |
| | 1.36 | 1.34 | 5.61 | 5.68 | | |
| 9. | 2.46 | 2.47 | 4.95 | 5.00 | 0.99 | 0.99 |
| | 2.46 | 2.47 | 4.94 | 4.99 | | |
| 10. | 1.42 | 1.43 | 4.87 | 4.98 | 1.00 | 0.98 |
| | 1.45 | 1.44 | 4.86 | 4.95 | | |
| Average | 1.24 | 1.22 | 4.99 | 5.10 | 1.01 | 0.98 |

**Table 2.** Correlation of Spinal Fluid Magnesium and Calcium Values by the Versene Titration and the Permanganate and Ammonium Phosphate Methods

| Spinal fluid | Magnesium | | Calcium | | Versene/$NH_4HPO_4$ | Versene/$KMnO_4$ |
|---|---|---|---|---|---|---|
| | Versene (mEq./L.) | $NH_4HPO_4$ (mEq./L.) | Versene (mEq./L.) | $KMnO_4$ (mEq./L.) | | |
| 1. | 2.0 | 2.2 | 2.3 | 2.4 | 1.10 | 1.05 |
| 2. | 3.3 | 3.2 | 2.9 | 2.9 | 0.97 | 1.00 |
| 3. | 1.8 | 1.8 | 2.4 | 2.5 | 1.00 | 0.98 |
| 4. | 2.1 | 2.1 | 2.9 | 2.9 | 1.00 | 1.00 |
| 5. | 1.7 | 1.6 | 2.1 | 2.1 | 1.06 | 1.00 |
| 6. | 1.3 | 1.4 | 2.2 | 2.4 | 0.93 | 0.92 |
| 7. | 3.1 | 3.1 | 2.3 | 2.3 | 1.00 | 1.00 |
| 8. | 2.7 | 2.9 | 2.7 | 2.6 | 0.93 | 1.04 |
| 9. | 2.6 | 2.6 | 2.4 | 2.5 | 1.00 | 0.96 |
| 10. | 2.6 | 2.6 | 2.1 | 2.0 | 1.00 | 1.05 |
| Average | 2.32 | 2.35 | 2.43 | 2.46 | 0.99 | 0.99 |

shown in Table 2. The slightly better correlation is probably due to the fact that the end-point is more clearly discernible in spinal fluid titrations, because of the almost complete absence of proteins and chromogenic substances which tend to mask the indicator colors in the titration of serum.

Recovery experiments were performed on both serum and spinal fluid. The results showed good recovery, between 100 and 106 per cent. The correlation of the Versene titrations with the two standard methods was 0.94 for serum, 0.96 for urine, and 0.98 for spinal fluid. The method for urine is quite long and tedious and is not recommended for routine laboratory procedure.

## CLINICAL APPLICATION

In an attempt to find some correlation between laboratory results and clinical entities, it was noted that in certain extreme conditions, such as terminal nephritis and diabetic coma, the normal ratio of magnesium to calcium in terms of mEq./L. appeared to be significantly altered. Accordingly, two sets of sera were analyzed for magnesium and calcium and the molar magnesium:calcium ratios were calculated. The first, or normal, set constituted the basis for the determination of the standard range of magnesium:calcium. An analysis of the literature dealing with normal values for serum calcium and magnesium revealed approximately the same range. The second set consisted of 100 pathologic sera, including repeated determinations on individuals comprising about 9 groups of systemic diseases. These were arbitrarily divided into 3 groups—those

**Table 3.** Statistical Analysis of Serum Magnesium and Calcium Values and the Mg/Ca Ratio in Normal and Pathologic Sera

| | *Normal* (N = 10) | *Pathologic* (N = 100) | | | |
|---|---|---|---|---|---|
| | | *Low* (21) | *Median* (43) | *High* (36) | *Total* |
| Magnesium mEq./L. | | | | | |
| Range | 1.00–2.46 | 0.00–1.24 | 0.89–2.49 | 1.85–4.32 | 0.00–4.32 |
| Average | 1.24 | 0.52 | 1.57 | 2.57 | 1.71 |
| S.E. | 0.10 | 0.10 | 0.13 | 0.09 | 0.09 |
| Calcium mEq./L. | | | | | |
| Range | 4.36–5.61 | 1.72–6.50 | 3.07–5.85 | 2.71–6.35 | 1.72–6.50 |
| Average | 4.99 | 5.04 | 4.26 | 3.61 | 4.19 |
| S.E. | 0.20 | 0.25 | 1.00 | 1.10 | 1.00 |
| Mg/Ca ratio | | | | | |
| Range | 0.22–0.50 | 0.00–0.21 | 0.22–0.48 | 0.51–1.65 | 0.00–1.65 |
| Average | 0.30 | 0.10 | 0.37 | 0.75 | 0.44 |
| S.E. | 0.06 | 0.04 | 0.09 | 0.13 | 0.10 |

**Table 4.** Mg/Ca Molar Ratios According to Normal and Abnormal Magnesium and Calcium Values

| *Ratio* | *Ca normal* | | | | *Ca abnormal* | | | | *Total* | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Mg normal* | | *Mg abnormal* | | *Mg normal* | | *Mg abnormal* | | | |
| | *No.* | *%* | *No.* | *%* | *No.* | *%* | *No.* | *%* | *No.* | *%* |
| Normal | 19 | 19 | .. | .. | 25 | 25 | .. | .. | 44 | 44 |
| Abnormal | 1 | 1 | 12 | 12 | 15 | 15 | 28 | 28 | 56 | 56 |
| Total | 20 | 20 | 12 | 12 | 40 | 40 | 28 | 28 | 100 | 100 |

with magnesium:calcium ratios below the standard range, those within the standard range, and those with values above the standard range. The results are shown in Table 3. The entire pathologic group was then arbitrarily divided again into 4 groups, as shown in Table 4.

The abnormal ratio obtained in the instance where both magnesium and calcium are normal (see Table 4) is due to the fact that the Mg/Ca ratios calculated from valves obtained from the literature, in which the ratios were calculated over the entire range of normal values, do not correspond to the observed ratios. The value in this case is 0.51, which may be considered on the borderline of the standard range, and thus may be excluded. We can then eliminate the first group from the discussion, since all of the ratios here are within the standard range. In the third group (Mg normal, Ca abnormal), we may say in general that the majority of this group have normal ratios, and that, while it may not detract from the clinical significance of the remainder of the group, patients with abnormally high or low calcium values generally fall under the clinical entities of nephritis, gastrointestinal disturbances, and parathyroid dysfunction. Over 50 per cent of the 25 patients in this group

**Table 5.** Normal and Abnormal Mg/Ca Ratios According to Systemic Groups

| *System or organ* | *Abnormal* | | *Normal* | |
|---|---|---|---|---|
| | *No.* | *%* | *No.* | *%* |
| Gastrointestinal | 13 | 56.5 | 10 | 43.5 |
| Renal | 7 | 70.0 | 3 | 30.0 |
| Endocrine | 7 | 63.6 | 4 | 36.4 |
| Hepatic | 7 | 50.0 | 7 | 50.0 |
| Biliary Tract | 6 | 85.7 | 1 | 14.3 |
| Cardiac | 5 | 29.4 | 12 | 70.6 |
| Gestational | 4 | 100.0 | .. | .. |
| Pulmonary | 4 | 80.0 | 1 | 20.0 |
| Hematologic | 2 | 100.0 | .. | .. |
| Averages | | 59.2 | | 40.8 |

fall into these three categories and may generally be diagnosed by more specific means.

Of the two remaining groups, which comprise 40 per cent of the entire pathologic series, neither shows normal ratios nor normal magnesium values; about 30 per cent of the groups have normal calcium values. It is of interest to note that whenever the magnesium value is abnormal, the Mg/Ca ratio is also abnormal, and while it may be difficult at the present time to correlate changes in serum magnesium values with disease, it may be possible to show some correlation of the ratio. Table 5 shows all the abnormal values (ratios) grouped according to systems, along with the number of normal values obtained in each disease group in the entire series.

## DISCUSSION

Clinicians, physiologists, and clinical chemists have long sought a relationship between serum magnesium concentration and various diseases with little or no success. A thorough search of the literature reveals only 1 instance in which the significance of the serum Mg/Ca ratio was examined. Jacoby and Friedel determined this value in experimental animals to which oxalic acid was administered both orally and parenterally. They found that, although there were extreme initial changes in the absolute concentrations of both magnesium and calcium, the normal ratio tended to be maintained over prolonged periods.

Of the 7 patients with nephritis who had abnormal ratios, 5, or half the total nephritic patients examined, had ratios higher than the standard range. In all the abnormal cases the deviations from the standard range were extreme, being 50 per cent or more.

Although the group with gastrointestinal diseases was heterogeneous, it may be said that they all had a common physiologic disturbance—decreased absorptive capacity in one region of the intestine or another. Of the 13 patients with abnormal ratios, 10, or 43 per cent of all patients in this series, had ratios above the standard range. The 3 instances of abnormally low ratios were in one individual, in whom extreme ascites had developed. Eight liters of abdominal fluid were removed at the first paracentesis.

Since the number of hematologic patients (multiple myeloma) is only 2, with neither showing an abnormal ratio, and since this disease is readily recognized by other laboratory and clinical examinations, the authors do not feel that these figures are significant.

Since, as has been pointed out by Rawson and Sunderman, the binding

powers of the serum albumin and serum globulin for calcium are approximately equal, and since this may very well be true also for magnesium, we cannot explain the abnormal ratios observed in liver disease on the basis of disturbances in albumin:globulin ratios. Equal numbers of normal and abnormal values were found. Since hepatic diseases are rarely uncomplicated, it seems likely that those cases in which the ratio is abnormal were possibly complicated by other factors, most probably by some intra- or extrahepatic biliary obstruction.

Of the total of 7 patients with biliary tract obstruction, 6 had abnormal ratios, and all of these fell below the standard range. The 1 normal value was from a patient who had formerly had a low ratio, and had meanwhile undergone a cholecystectomy. It is significant that no deaths, serious complications occurred in any of these cases, as compared with the deaths in the nephritis and gastrointestinal groups.

The number of cardiac patients with abnormal ratios was 5, or about 29 per cent of the total cases in the series. The 12 standard values covered the entire standard range, with an average value of 0.38, which approximates the average of the entire median group. The average of the entire series of cardiacs is 0.46, which approximates the average of the entire series. It is therefore apparent that these results are not clinically significant.

Four abnormal values were observed in pulmonary involvement, all above the standard range. The single case giving a normal value was one of tuberculosis. This value was 0.43, approximating the average of the entire series. While it is difficult to ascertain the physiologic causes of variations of the Mg/Ca ratio in these instances, it may be pointed out that in emphysematous conditions great difficulty is experienced in breathing, i.e., in gaseous exchange. This usually leads to secondary phenomena, particularly tissue anoxia, which may bring about reversible or irreversible changes in cell membrane permeability and selectivity, which may account for the redistribution of magnesium and calcium throughout the body.

The ratios obtained in all patients in advanced pregnancy in which toxemia or edema had developed were abnormal; nevertheless, the majority of the ratios were not significantly removed from the standard range. A value of 1.32 was obtained in 1 patient who had severe hypoproteinemia, and the calcium value had fallen to an extremely low level. It is quite possible, however, that the ionized or physiologically active portion remained relatively unaffected.

Although 64 per cent of all endocrine disorders in this series were

associated with abnormal ratios, nevertheless, it is felt that these diseases—diabetes, hypoparathyroidism, and hypothyroidism—can be diagnosed more readily by other laboratory and clinical methods.

## SUMMARY

1. The Schwartzenbach method has been adapted for the simultaneous determination of calcium and magnesium in serum and spinal fluid. The method appears to be adaptable to all body fluids, including thoracic and abdominal transudates and exudates. Its use in the clinical laboratory is recommended because of the great saving of time, particularly in the determination of magnesium.

2. A series of 100 pathologic cases of various clinical entities has been analyzed by the new method and checked with the standard methods. A calculation and statistical analysis has revealed the Mg/Ca molar ratio to be of possible clinical significance.

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the Clinical Chemist

## EDITORIAL

The American Association of Clinical Chemists has promulgated a policy on legislation in a Resolution passed by the National Executive Committee in 1950 and reaffirmed three years later (1). These tenets still hold, and they were proposed in the best interest of those who require the services of clinical chemistry.

In this Resolution one of the clauses stipulates that the chemist should not be required to show proficiency in fields other than clinical chemistry to be permitted to practice his specialty. It is our conviction that disciplines such as hematology, microbiology, serology, and other such allied specialties, are separate sciences in spite of any overlap in the periphery. That they are frequently housed close to the chemistry laboratories, or even grouped in one laboratory, is fortuitous and the result of their historical development in the hospital.

The Attorney General in California has recently published an opinion (2) that "a technologist's license may not be issued to anyone not conforming to the requirement of five years general experience." This general experience refers to all the specialties above mentioned. We respectfully take issue with the Attorney General in California and should furthermore like to point out that real progress will be made by specialists in the respective disciplines and not by technologists whom his definition makes jacks-of-all-trades.

The American Association of Clinical Chemists is making every effort to attract competent chemists into our specialty. This will be most difficult if in the

process it will first be necessary to train chemists as medical technicians. Should the California opinion become generally accepted the science of clinical chemistry and all who are dependent on its services will have received a serious setback.

MAX M. FRIEDMAN
*National Secretary*

## REFERENCES

1. *Clinical Chem.* **5**, 65 (1953).
2. Opinions of the Attorney General. Hanna Legal Publications, Albany, Calif., **24**, 79 (1954).

## FEDERATION MEETINGS: APRIL 10–16

The American Society of Biological Chemists, through Dr. Elmer Stotz, Program Chairman, has invited the American Association of Clinical Chemists to arrange a symposium on "Biochemistry of Disease" for the Federated Societies Meeting in San Francisco, April 10–16.

Dr. Marschelle H. Power has arranged the symposium for the A.A.C.C. The program is as follows:

"Porphyrins and Porphyrin Precursors in Human and Experimental Porphyria"—S. Schwartz, University of Minnesota, Medical School.

"Investigations of Diseases of the Thyroid Gland With Radioactive Iodine"—C. A. Owen, Jr., Mayo Clinic.

"Biochemical Studies in Relation to Comparative Susceptibility to Experimental Atherosclerosis"—L. Pilgeram, University of Illinois, College of Medicine.

"The Clinical Chemical Significance of Ionography"—H. J. McDonald, Stritch School of Medicine, Loyola University.

"Observations on Aldosterone In Human Urine"—J. A. Luetscher, Jr., School of Medicine, Stanford University.

## MEMBERSHIP STATISTICS

The American Association of Clinical Chemists was founded by nine charter members on December 15, 1948. Since that date there has been a continuous increase in the membership. Table 1 is a summary compiled from the last five directories, including the 1955 Directory published in March, and contains a tabulation of the total members listed according to the years in which the individuals were admitted into the Association. In the past six years 16 members have died and 81 others have either resigned or been dropped for nonpayment of dues. The last year, 1954, saw an encouraging addition of new members, being only surpassed by 1949 and 1950. The steady increment over the years is evidence that the Association has yet to reach its full strength.

**Table 1.** Distribution of Members in the American Association of Clinical Chemists as Tabulated from the Directories of the Designated Dates. Listings Are According to the Year of Admission to Membership

| | 10/15/50 | 1/1/52 | 1/1/53 | 1/15/54 | 1/30/55 |
|---|---|---|---|---|---|
| 1948 | 9 | 8* | 8 | 8 | 8 |
| 1949 | 166 | 154 | 152 | 129 | 121 |
| 1950 | 134 | 172 | 170 | 156 | 140 |
| 1951 | | 81 | 75 | 69 | 65 |
| 1952 | | | 71 | 69 | 65 |
| 1953 | | | | 83 | 81 |
| 1954 | | | | 8 | 137 |
| 1955 | | | | | 12 |
| Totals | 309 | 415 | 476 | 522 | 629 |

* Jos Kahn, a charter member, died on December 30, 1951.

**Table 2.** Geographical Distribution as Percentages of Total Tabulated from the Directories of the Designated Dates

| | 10/15/50 | 1/1/52 | 1/1/53 | 1/15/54 | 1/30/55 |
|---|---|---|---|---|---|
| N. Y. | 28.2 | 25.1 | 22.9 | 21.2 | 19.0 |
| Penn. | 9.1 | 9.4 | 9.2 | 9.9 | 10.3 |
| Calif. | 8.4 | 10.1 | 10.5 | 10.3 | 9.2 |
| Ill. | 8.4 | 9.4 | 8.0 | 7.8 | 7.0 |
| Mass. | 11.3 | 9.6 | 9.5 | 7.7 | 6.6 |
| Iowa | 1.6 | 2.4 | 1.7 | 4.0 | 4.6 |
| Md. | 1.3 | 1.9 | 2.9 | 4.0 | 4.4 |
| N. J. | 3.2 | 3.6 | 4.6 | 4.2 | 3.5 |
| Ohio | 3.2 | 3.1 | 2.9 | 2.7 | 3.0 |
| D. C. | 1.0 | 1.2 | 1.9 | 2.9 | 2.8 |
| Tex. | 1.3 | 1.9 | 1.9 | 1.7 | 2.8 |
| Mich. | 2.9 | 2.7 | 2.9 | 2.1 | 2.5 |
| Mo. | 2.9 | 2.4 | 2.3 | 2.3 | 2.5 |
| Other | 17.2 | 17.1 | 17.0 | 18.9 | 21.6 |
| Total | 309 | 415 | 476 | 522 | 629 |

Table 2 shows the geographic distribution in percentage of total membership as tabulated from Association files. This table may be confined to the "department of useless information" except that it bears out the fact that where a local section exists the membership is significantly higher than where there is none.

## IN MEMORIAM

Adolph Bernhard, 64, director of the Biochemical Laboratories at the Lenox Hill Hospital in New York City, died at the hospital on December 1, 1954 after an extended illness.

He received a B.S. from the Polytechnic Institute of Brooklyn and an M.A. from Columbia University in 1922. He was appointed chief biochemist to Lenox Hill Hospital in 1915 and served there continuously until his death.

He was a member of the American Chemical Society, Sigma Xi, The Society for Experimental Biology and Medicine, the New York Academy of Sciences, and was a Fellow of the American Association for the Advancement of Science. He had published over 60 papers in clinical biochemistry.

Adolph Bernhard was one of the first to be admitted to membership in the American Association of Clinical Chemists and was active in the organizational stages. At that time he made available to his colleagues the benefit of his long experience in clinical chemistry. He was invited to lecture in Glasgow in October, 1954, to the Scotland and Northern Ireland Region of the British Association of Clinical Biochemists, but could not accept due to ill health.

He is survived by his wife and a daughter.

## AMERICAN BOARD OF CLINICAL CHEMISTRY

The American Board of Clinical Chemistry has authorized preparation and distribution of a *Directory of Certified Clinical Chemists*. This directory is now being distributed to chemists listed therein and to numerous university departments of chemistry and biochemistry. Heads of hospital departments or other laboratories engaged in clinical chemistry can secure a copy of the Directory by writing to the Secretary-Treasurer, Dr. O. H. Gaebler, Henry Ford Hospital, Detroit 2, Michigan.

A copy of the *Instructions to Applicants for Certification*, as approved by the Board at its annual meeting in 1954, is also available without cost. Chemists wishing to apply for certification should secure this information at once, since considerable time is required to process a formal application.

## ASSOCIATION NOTES

### Membership Certificates

Members and associate members may obtain a membership certificate that is suitable for framing. Anyone interested in ordering same should send a check for four dollars to the National Treasurer, Dr. Louis B. Dotti, St. Luke's Hospital, New York 25, N. Y. Please state how you wish your name inscribed and the degrees to be included.

### Membership Requirements

Information concerning requirements for membership in the Association may be obtained by writing to the National Secretary, Dr. Max M. Friedman, Lebanon Hospital, New York 57, N. Y. Present subscribers to *Clinical Chemistry* will have subscription included in 1956 dues.

### Members in Arrears

Members who have not paid their annual dues by March 15 will be removed from the membership roster. In the past a longer period of grace was permitted, but with our commitments for the journal this cutoff date is necessary. However, for this year only, those still in arrears will be reinstated if payments are received before May 1, 1955.

### Ballots

Members of the Association have received a directory and a ballot for the election of officers to the Executive Committee of 1955–56. These ballots must be returned to the National Secretary by May 1, 1955, to be counted in the final tabulation.

### Local Sections

There are at present seven chartered local sections in the Association. These include, in the order of their formation, Metropolitan New York, Boston, Philadelphia, Southern California, Chicago, Washington-Baltimore-Richmond, and Midwest. Other areas are now in the process of organizing such local sections, and Greater Kansas City has already applied for section status. All local sections are invited to submit copy of news of their activities for publication in CLINICAL CHEMISTRY.

## REPORTS FROM THE SECTIONS

### Metropolitan New York

*A. Saifer*

The Section held a successful symposium and exhibit on "Recent Advances in Instrumentation" during January 21 and 22, 1955, at the New York Medical College. A symposium on "Physical Methods in Clinical Chemistry" was attended by an overflow audience of more than 400 people. The speakers included Robert J. Slater of Cornell University Medical College on "Paper and Zone Electrophoresis"; Charles L. Fox, Jr. of the New York Medical College on "Flame Photometry"; and Solomon A. Berson of the Bronx V. A. Hospital on "Radioisotopes as Tracers." Kurt G. Stern of Polytechnic Institute of Brooklyn was chairman.

A "Round Table on Instrumentation" was held the following morning. The participants included Joseph Greenspan, Process and Instruments, Brooklyn, N. Y.; Sergei Feitelberg, Mt. Sinai Hospital, N. Y.; and Alfred Henley, American Instrument Company, Silver Spring, Md. The subjects covered were flame photometers, radioisotope equipment, and electrophoresis instrumentation. Julius J. Carr of the Mount Sinai Hospital was chairman.

More than 30 companies participated in the exhibits. Space does not permit the mention of all whose efforts made this possible. Special thanks should be extended to the New York Medical College for making its facilities available, and to William Gruen of Instrumentation Associates for carrying the major efforts in organizing the exhibits.

### Washington-Baltimore-Richmond

*Marian E. Webster*

The first meeting of the 1954–55 year was held on November 18, 1954, at the Walter Reed Army Medical Center. A symposium on measurements of thyroid function and their interpretation was the topic for the evening.

Recent advances in the analysis of protein bound iodine was discussed by Max Sherer, Cancer Institute of the National Institutes of Health. James C. Syner, Walter Reed Army Hospital, could not be present to talk on the measurement and evaluation of basal metabolic rate data, but the abstract of his paper was read by Lt. Col. Irving Gray and commented on by J. I. Sherman. Herschel J. Wells concluded the symposium with a discussion of the use of $I^{131}$ uptake in the measurement of thyroid function.

The second meeting of the year was scheduled for January 6, 1955, and was devoted to a discussion of recent advances in the laboratory control of diabetes mellitus. The third meeting was held on March 3, at which hematologic technics and their interpretation was discussed. The final meeting will be held on April 21. The program will be announced.

The American Instrument Company, A. S. Aloe Company, and Fisher Scientific Company have generously contributed funds to the Washington-Baltimore-Richmond Section in order to assist in the scientific program for 1955. These funds assure the success of the scientific meetings.

## Southern California

*Clyde A. Dubbs*

"The (California) Clinical Laboratory Technology Act does not provide for the licensing of technologists in special fields." This opinion, rendered August 27, 1954, by the Attorney General of California, means that the clinical chemist is still legally restrained from directing a clinical chemistry laboratory unless he also has additional general experience in all the clinical laboratory fields.

The law now makes no specific provision for *special technologists* who can direct laboratory work in their special fields, although Section 1264 of the Act does provide that "licenses under this chapter may cover work in any one basic science, or may cover proficiency in the entire field of clinical laboratory work." Nevertheless the Attorney General stated that this "general provision of Sec. 1264 is in conflict with Sec. 1260, which requires that technologists have five years of experience embracing the various fields."

It is believed that this conclusion does not conform to the intentions and expectations of clinical chemists and others who supported the 1951 law.

The first symposium of the current year was held November 2, 1954, at the Hollywood Presbyterian Hospital. The subject, "Calcium and Phosphorus Metabolism in Relation to Bone Disorders," was discussed by William R. Bergren, George N. Donnell, and Mary Mulloy, staff members of the Childrens Hospital and the University of Southern California Medical School.

The second symposium, on the "Determination, Origin and Significance of Corticosteroids" was held on December 7 at the Cedars of Lebanon Hospital, Los Angeles. The speakers were Robert Commons, University of Southern California Medical School; Orville J. Golub, Bio-science Laboratories; and Harry Sobel, Cedars of Lebanon Hospital.

A special dinner on January 4, 1955, at the Carolina Pines Restaurant was devoted to a discussion of the California Attorney General's opinion on specialized technologist licensure and its consequences; and to discussion of the inter-laboratory accuracy survey program of the Southern California Section.

# LETTER to the editor

*To the Editor:*

In *The Clinical Chemist 6:* 61 (1954) Henry J. Goeckel of Cranford, N. J., asks our organization to give serious consideration to the development of comprehensive, reliable standard specimens for the specific use of the average institutional and community laboratory for checking the accuracy of their biochemical procedures. He also suggests that such standards be assayed by a sufficient number of our large research-type institutional laboratories to establish the limits of accuracy of the *Standard Methods* and that such standard samples be readily available to any interested laboratory.

The inconsistency of publishing a book of standard methods (with more to follow) without making available standard analyzed samples, is a problem that we in the New York Section have wrestled with over the past two years. We feel that we have arrived at a partial solution to this important problem by the use of sterile bovine ultrafiltrate as a general laboratory standard. This is a water-clear solution which contains all the constituents of bovine blood (except proteins and sterols) at approximately their original concentrations and, unless contaminated, it remains stable over many months under ordinary refrigeration and use. It is treated exactly as if it were a blood specimen and reports from some 12 laboratories scattered over the country have given excellent reproducibility of values with a variety of methods and instruments. As soon as a few additional results are achieved, we are planning to make a complete statistical analysis of the results and to publish them in CLINICAL CHEMISTRY.

While it is our hope then to make these samples available through various laboratory supply dealers, it should be stressed that we are not endorsing the products of any manufacturer or dealer. In fact, it is our purpose and function to check and evaluate all such proposed standards. We are at the present time looking into the matter of testing some protein- and sterol-containing standards which have been recently brought to our attention, since these constituents are not covered by the ultrafiltrate standard.

While this report is somewhat premature, we feel that our readers should be informed that our organization is taking constructive steps in attempting to improve the accuracy of clinical laboratory procedures.

Sincerely yours,

ABRAHAM SAIFER

*Secretary Metropolitan-New York Section*

# ABSTRACTS

*Editor:* Ellenmae Viergiver. *Contributors:* Joseph A. Annino, Gladys J. Downey, Clyde A. Dubbs, Alex Kaplan, Margaret M. Kaser, Miriam Reiner, Herbert Thompson

**A method of insulin bio-assay and its application to human plasma fractions.** F. C. Goetz, P. M. Beigelman, and G. W. Thorn (*Harvard Medical School and Peter Bent Brigham Hospital, Boston, Mass.*).

Concentrated human plasma protein preparations were assayed for insulin activity by a modification of the method of Yenerman, Cornfield, Bates, and Anderson [*Federation Proc.* **12,** 162 (1953)]. Hypophysectomized, alloxanized male mice were given the test material by intraperitoneal injection with simultaneous administration by gavage of 300 mg. dextrin. Blood glucose was determined by the Nelson-Somogyi procedure [*J. Biol. Chem.* **153,** 375 (1944); **195,** 19 (1952)] in 0.02 ml. samples collected after 4, 30, and 65 minutes. Near-complete suppression of the hyperglycemia was produced by 4–5 milli-units of insulin with equivocal results with 1–2 milli-units. No insulin activity could be demonstrated in 0.25–1.0 ml. of human plasma, but it was present in 0.25 ml. or more of a 5-fold concentration of globulin.—*Proc. Soc. Exp. Biol. Med.* **86,** 484 (1954).

M.K.

**Electrophoretic fractionation of serum protein in multiple sclerosis.** J. Bernsohn and L. S. G. Cochrane (*Veterans Administration Hospital, Hines, Ill.*).

Electrophoretic separations of the serum proteins of 27 patients with multiple sclerosis were carried out for 70–80 minutes in a Tiselius apparatus in Veronal buffer, *p*H 8.6 and 0.1 ionic strength, after dialysis for 24 hours against the buffer at 3° and with a protein concentration of approximately 1 per cent. Area and mobility measurements were made on the ascending boundary. Protein determinations were made by the micro-Kjeldahl method. There was no difference between the mean total serum protein concentrations of the patients and of 16 normal subjects, but the albumin fraction averaged 55 per cent in the multiple sclerosis cases and 60.2 per cent in the controls, while the mean figures for $\alpha_2$-globulin were 12.4 per cent and 8.9 per cent, respectively. These differences were statistically significant ($p < 0.001$). There were no significant quantitative variations in the other globulin components. In 15 patients the $\alpha_2$-globulin exhibited a double peak.—*Proc. Soc. Exp. Biol. Med.* **86,** 540 (1954).

M.K.

**Aluminum hydroxide as an adsorbing agent for urinary gonadotropins** R. F. Malburg and J. R. Goodman (*Paraplegia Laboratory, Veterans Administration Hospital, Long Beach, Calif.*)

A procedure is described for adsorbing urinary gonadotropins with aluminum hydroxide. The reagents are inexpensive and commonly available and the method is simple and rapid. Results obtained with this procedure were in agreement with those obtained with the usual alcohol-precipitation method.—*J. Clin. Endocrinol. & Metab.* **14,** 666 (1954).

E.V.

**Jaundice and xanthochromia of the spinal fluid.** L. B. Berman, L. W. Lapham, and E. Pastore (*Medical Services and Neurological Service, Boston City Hospital; Dept. of Medicine and Dept. of Biochemistry, Boston University Medical School; and Dept. of Neurology, Harvard Medical School; Boston, Mass.*)

Bilirubin concentration was determined in serum and in spinal fluid from 20 jaundiced patients. Detectable amounts were present in the spinal fluid, including those specimens from patients with jaundice of recent onset. The concentration in the spinal fluid ranged from 1:10 to 1:100 of the serum values. Although the data did not show a linear relationship between serum and spinal fluid bilirubin, there was a trend in that direction, the higher spinal fluid values occurring when the serum values were high.—*J. Lab. & Clin. Med.* **44,** 273 (1954).

E.V.

**The use of glycogen as a substrate for the determination of serum amylase.** H. S. Friedman (*Tampa Municipal Hospital, Tampa, Fla.*)

Glucose liberated from a buffered glycogen substrate is measured in a barium zinc filtrate by the Somogyi-Nelson copper reduction method. The procedure is said to be simple, rapid, and accurate. The glycogen substrate is more stable than starch substrate and is easier to prepare.—*J. Lab. & Clin. Med.* **44,** 308 (1954).

E.V.

**Blood levels of absorbed labeled fat and chylomicronemia.** W. W. Burr, Jr., C. Dunkelberg, J. C. McPherson, and H. C. Tidwell (*Southwestern Med. School, Univ. of Texas, Dallas, Tex.*)

Chylomicron counts were compared with the blood levels of absorbed fat containing palmitic acid-1-$C^{14}$. The similarity of the results obtained by these two methods suggests that the same function is being measured. Thus, chylomicron counts and the areas under the curves from them may be used as a satisfactory relative measure of the changing blood levels of absorbed fat and as a means of following the active process of fat absorption.—*J. Biol. Chem.* **210,** 531 (1954).

E.V.

**A method for the estimation of inulin in plasma and urine containing dextran.** J. R. K. Preedy (*Coll. of Physicians & Surgeons, Columbia Univ., and Presbyterian Hosp., New York, N. Y.*)

A modification of the method of Roe et al. (*J. Biol. Chem.*, **178,** 839 [1949]) is presented. Proteins in plasma or urine diluted to contain 15–55 mg. of inulin are precipitated with zinc chloride and sodium hydroxide. Dextran is precipitated from the protein-free filtrate by ethanol. The dextran-free supernatant is evaporated to dryness. The residue is treated with resorcinol-thiourea reagent and hydrochloric acid solution, and the color intensity compared with standard inulin solutions.—*J. Biol. Chem.* **210,** 651, (1954).

E.V.

**The determination of 17,21-dihydroxy-20-ketosteroids in urine and plasma.** R. H. Silber and C. C. Porter (*Merck Inst. for Therapeutic Research, Rahway, N. J.*)

A procedure is described which is applicable to routine clinical use, and which can be completed within a working day. It involves (a) a 15-second extraction with chloroform, (b) a 10-second washing with dilute alkali, (c) a 15-second extraction of an aliquot of the chloroform extract with alcoholic phenylhydrazine-sulfuric acid reagent, and (d) measurement of the optical density in microcuvettes in the Beckman spectrophotometer after heating the samples to 60° for 30 min. Concentrations as low as 6 μg. per 100 ml. can be determined.—*J. Biol. Chem.* **210,** 923 (1954).

E V.

**A rapid colorimetric method for the determination of isonicotinic acid hydrazide in blood plasma: II. Application to blood plasma from human tuberculosis patients.** B. Prescott, S. Katz, and G. Kauffman (*U.S. Dept. of Health, Education, and Welfare, Public Health Serivce, Natl. Inst. of Health, Natl. Microbiological Inst., Bethesda, Md., and D.C. General Hosp., Washington, D.C.*)

A simple and rapid procedure for the determination of isoniazid in blood plasma is presented which employs 4-pyridylpyridinium dichloride as indicator.—*J. Lab. & Clin. Med.*, **44,** 600 (1954).

E.V.

**A rapid method for the estimation of morphine.** J. M. Fujimoto, E. Leong Way, and C. H. Hine (*Sch. of Med. and Pharmacy, Univ. of California, San Francisco, Calif.*)

A method is described which is highly specific, requires no specialized apparatus, and can be carried out in less than 90 min. The morphine is extracted from a highly alkalinized solution with *n*-butanol and interfering substances are re-

moved in subsequent extraction steps. A colorimetric phenol reagent is used for the final estimation in a buffer extract. Optical density is measured at 675 mμ.—*J. Lab. & Clin. Med.*, **44**, 627 (1954).

E.V.

**Paper electrophoresis with automatic scanning and recording.** G. R. Cooper and E. E. Mandel, with technical assistance of R. H. Owings and J. Fetner (*U.S. Dept. of Health, Education & Welfare, Emory Univ. Sch. of Med., and Grady Memorial Hosp., Atlanta, Ga.*)

An automatic scanning and recording apparatus is described which furnishes electrophoretic patterns closely comparable to those obtained by means of the standard Tiselius apparatus.—*J. Lab. & Clin. Med.* **44**, 636 (1954).

E.V.

**Blood ACTH in man: "active" and "activable" fraction.** G. Morruzzi, C. A. Rossi, L. Montanari, and M. Martinelli (*Univ. di Bologna, Italy*)

Blood ACTH has been assayed in 6 normal subjects and in 6 patients with hypocorticism, a condition reported to have an increased level of blood ACTH. Two different technics were used: (1) the direct method of injecting plasma directly into hypophysectomized rats and assaying ACTH by the adrenal ascorbic acid depletion method; (2) mixing the plasma with 4 volumes of glacial acetic acid and processing with oxycellulose, the eluate being assayed as above. Two fractions of ACTH have been found in human blood. One, called "active" ACTH, is a labile and ultrafiltrable fraction which is found mainly in normal subjects. The other, called "activable" ACTH, is detectable only after treatment with oxycellulose. This form occurs mainly in the blood of human subjects with hypocorticism, but is present normally in the blood of other species of animals. —*J. Clin. Endocrinol. & Metab.* **14**, 1144 (1954).

M. R.

**Indirect method for determination of serum inorganic sulfate by flame spectrophotometry.** R. D. Strickland and C. M. Maloney (*Veterans Administration Hospital, Albuquerque, N. M.*)

Serum inorganic sulfate determination is of value as an early indication of renal insufficiency because significant increase above the normal range of 0.3–1.0 mEq./l. may occur before any retention of inorganic phosphate, blood urea nitrogen, or creatinine can be detected. To 9 ml. of uranyl acetate solution (4 Gm. $UO_2Ac_2 \cdot 2H_2O$/l.) in a 15-ml. conical bottom centrifuge tube, add 3 ml. of serum. Cover the tube with Parafilm and mix thoroughly by shaking. Centrifuge and filter the supernatant liquid. Pipette 4 ml. of the filtrate into a test tube and add 1 ml. of solution containing 5 mEq. $BaCl_2$/l. (20 mEq./l. if a very high sulfate determination is anticipated). Prepare a blank using 2 ml. of filtrate and 0.5 ml. of water to correct for interfering substances determined as "barium."

Let stand 1 hour and filter to remove $BaSO_4$. The barium remaining in the sample and in the blank is determined with the Beckman B Spectrophotometer with oxygen (15 lb./sq. in.) and acetylene (1.5 lb./sq. in.) at 873 mμ and slit width of 1.0 mm. From a standard curve determine barium and compute mEq. of sulfate as the difference between mEq. of barium added and the amount remaining in the filtrate after sulfate precipitation. The standard curve is prepared following this procedure and using a standard sodium sulfate solution (20 mEq./l. so diluted that a series of solutions varying in concentration from 0.0 to 20 mEq./l. are obtained. Time required is approximately 90 minutes and the error is small. In advanced renal insufficiency, inorganic sulfate may reach 20 mEq./l.—*Am. J. Clin. Pathol.* **24,** 1100 (1954).

H. E. T.

**A spectrophotometer adapter for reading paper strips.** D. M. Schultz, R. L. Dryer, and M. A. Holdcraft (*Indiana University School of Medicine, Indianapolis, Ind.*

Details and illustrations are presented for the construction of an adapter for reading paper strips obtained in electrophoresis or chromatography with the Coleman Junior spectrophotometer. The device could be used with slight modification on other instruments of a similar type. With this adapter inserted in the cuvette well, the spectrophotometer becomes a satisfactory and convenient substitute for a densitometer. The curves obtained have been quite comparable to those derived by other methods of direct photometry and, in general, lend themselves easily to planimetry.—*Am. J. Clin. Pathol.* **24,** 1110 (1954).

H. E. T.

**Barbiturate studies: II. Correlation between clinical condition and blood barbiturate levels.** I. Sunshine and E. R. Hackett (*School of Medicine, Western Reserve University, Cleveland, Ohio*).

Blood barbiturate levels in patients who had received excessive dosages were determined by the procedure of Goldbaum [*Anal. Chem.* **24,** 1604 (1952)] and correlated with the patients' clinical condition. For phenobarbital the blood levels associated with varying stages of drug effect were: Stage I, awake, competent, and mildly sedated, 2.4–3.6 mg./100 ml.; Stage II, sedated, reflexes present, prefers sleep, 4.2–4.6 mg./100 ml.; Stage III, comatose, reflexes present, 5.0–6.2 mg./100 ml.; Stage IV, comatose, areflexive, 7.7–9.2 mg./100 ml.; Stage V, comatose, circulatory and/or respiratory difficulty, 20.6 mg./100 ml., with death of the patient (1 case). For phenobarbital, decrease in the blood level to one half the original level requires 3 days. Data are also given for secobarbital, amobarbital, butabarbital, and pentobarbital, in which the blood levels of barbiturate in each stage of the drug effect are all lower than for phenobarbital. *Am. J. Clin. Pathol.* **24,** 1133 (1954).

H.E.T.

**Application of densitometry to paper chromatography of barbiturates.** A. J. McBay and E. J. Algeri (*Harvard Medical School, Boston, Mass.*).

Using the Beckman DU spectrophotometer, densitometry was successfully applied to paper chromatograms of the location and identification of barbiturates. The method has been applied to chromatograms of extracts of stomach contents, liver, blood, and urine, as well as to those of pure materials.—*Am. J. Clin. Pathol.* **24,** 1139 (1954).

H.E.T.

**Transphosphyralation as a source of error in assay of serum acid phosphatase.** R. B. Barrueto, M. M. D. Reynolds, B. S. Walker, and H. M. Lemon (*Boston University School of Medicine, Boston, Mass.*).

In a study of the method of Andersch and Szcypinski [*Am. J. Clin. Pathol.* **17,** 571 (1947)], in which the substrate used is nitrophenylphosphate, a small transfer of phosphate was found to take place. However, this does not affect the usefulness of the method as a clinical laboratory test.—*Am. J. Clin. Pathol.* **24,** 1144 (1954).

H.E.T.

**Two methods for the determination of glycogen in liver.** J. van der Vies (*N. V. Organon, Oss, Holland*).

A 200 mg. sample of liver is homogenized in 20 ml. of 5% trichloroacetic acid. The glycogen content may be determined rapidly by adding 3 ml. of Lugol solution to 2 ml. of liver extract and reading the optical density at 650 mμ. The amount of glycogen is read from a calibration curve, which must be prepared using glycogen of the same origin as the sample to be estimated.

The glycogen may be determined more accurately by hydrolyzing 2 ml. of the above liver extract with 2 ml. of 10N KOH at 100° for 1 hour. After cooling, 1 ml. of glacial acetic acid is added to neutralize the alkali and the solution is diluted to 20 ml. Two milliters of this solution is added slowly to 4 ml. of anthrone reagent (0.5 Gm. anthrone dissolved in 250 ml. of 95% (W/V) $H_2SO_4$ in a test tube placed in cold water. Color is developed by heating at 100° for 10 minutes. After cooling, the density is read in a photometer within 2 hours.—*Biochem. J.* **57,** 410 (1954).

A.K.

**Influence of smoking on urinary pepsinogen excretion.** S. Bornstein and S. Eichen (*Veterans Administration Hospital, Montrose, N. Y.*).

Urinary pepsinogen was determined by the method of West, Ellis, and Scott [*J. Lab. Clin. Med.* **39,** 159 (1952)] with a casein substrate [Goodman, Sandoval, and Halstead, *J. Lab. Clin. Med.* **40,** 872 (1952)]. Twenty white male office and professional hospital workers between 18 and 45 years of age, who smoked 20 or more cigarettes per day, were each examined 5 times. The control group consisted of 20 nonsmokers. Considerable diurnal and day-to-day variation was

found in the controls. The average urinary excretion of pepsinogen for the smokers was 43 units per hour compared with a value of 30 for the controls, a difference which had a $p$ value of 0.03 by the "t" test. A group of 5 smokers had an average excretion of 47 units per hour when they smoked during tests as compared with 43 units when they abstained. No information is given concerning the length of the experimental periods, but the statements are made that the immediate effect of smoking, although of statistical significance, is not of appreciable magnitude and that there is a prolonged effect on pepsin secretion.—*Proc. Soc. Exp. Biol. Med.* **86,** 619 (1954).

M.K.

**Conjugated steroids: V. Hydrolysis of total ketosteroids in urine.** I. B. Oneson and S. L. Cohen (*University of Minnesota Medical School, Minneapolis, Minn.*)

An improved method of hydrolysis is presented which results in an increase of about 15 per cent in the total steroids of urine as compared with hydrochloric acid hydrolysis. The hydrolysate contains larger amounts of ketosteroid sulfates and about 20 per cent of the total steroids as the beta type. The procedure is similar to that previously described for ketosteroid sulfates [Cohen and Oneson, *J. Biol. Chem.* **204,** 245 (1953)]. Acidified urine ($p$H 2–3) is extracted with butyl alcohol. The extract is washed with water and evaporated to dryness at a temperature not exceeding 60°. Portions,equivalent to 100–200 ml. of urine are hydrolyzed by heating for 10 minutes on a steam bath with 10 ml. of 1,4-dioxane. containing 10% of concentrated hydrochloric acid.—*Proc. Soc. Exp. Biol. Med.* **86,** 728 (1954).

M.K.

# BOOK REVIEWS

**Organic Analysis (Vol. 2)**

J. MITCHELL, JR., I. M. KOLTHOFF, E. S. PROSKAUER, AND A. WEISSBERGER (Eds.) Interscience Publishers, Inc., New York, 372 pp., $8.50

This is the second of the excellent series, *Organic Analysis*, designed to present reliable and critical information on general methods of direct analysis of organic systems. It stresses chemical and instrumental technics for analysis through functional groups.

The topics of Volume 2, and their authors, are:

"Microdetermination of Carboxyl Groups (Neutralization Equivalent)," by Al Steyermark.

"Determination of Esters," by R. T. Hall and W. E. Shaefer.

"Determination of Nitro, Nitroso and Nitrate Groups," by W. W. Becker and W. E. Shaefer.

"Application of Lithium Aluminum Hydride to Organic Analysis," by T. Higuchi.

"Coulometric Methods," by W. Donald Cooke.

"Application of Polarography to Organic Analysis," by Philip J. Elving.

"Methods Based on Reaction Rate," by T. S. Lee.

"Phase Solubility Analysis," by W. J. Mader.

"Countercurrent Distribution," by J. R. Weisiger.

This volume also contains the indices to Volume I and II.

**Legal Medicine, Pathology and Toxicology (ed. 2)**

T. A. GONZALES, M. VANCE, M. HELPERN, AND C. J. UMBERGER. Appleton-Century-Crofts, Inc., New York, N. Y., 1349 pp., $22.00

This noted text and reference book has been revised and brought up to date in this enlarged second edition, which contains almost twice the number of pages than the original, first published in 1937. As before, the text is well illustrated with photographs of actual cases taken from the files of the Office of the Chief Medical Examiner of New York City. The statistical data incorporates cases through 1951.

The first 40 chapters are devoted to pathology as applied to this type of work. New subject matter has been added to include autopsy findings in enbalmed bodies, cardiac contusions, sports injuries, serologic applications (Rh-Hr factors), radioactive compounds, new drugs, poisonings, and operative and postoperative deaths.

The entire section, 365 pages, on analytic toxicology has been rewritten. This section contains much data and formerly unpublished methodology of the Microchemical-Physical Laboratory of the Medical Examiner's Office. The

hospital chemist would have to critically evaluate the methodology for application to the needs of an active hospital laboratory. In the main, the methodology and instrumentation are applicable to large state and city laboratories dealing chiefly with such analytical problems, who have enough funds for the purchase of the specialized instrumentation. In isolated portions of this section, the chemist can find the rapid and simple chemical procedures that one could apply to the occasional requests from the emergency wards of the general hospitals, but the major portion of the procedures enumerated are too time consuming and require apparatus not usually found in hospital laboratories. On reading this section, one is left with the feeling that there is great need for a critical compilation of the fundamental, rapid, and specific methodology for use as part of the toxicologic procedures encountered in an active hospital chemistry laboratory. Here, speed and accuracy are essential for the immediate treatment of the patient.

As a reference book for pathologists and chemists concerned with forensic toxicology this text is essential to the laboratory library. The clinical chemist, with careful adaptation of the published procedures can establish an efficient routine which can be of practical help to his toxicologic problems. The descriptive toxicology (diagnosis and treatment), is unexcelled.

**Electrometric pH Determinations: Theory and Practice**

ROGER G. BATES. John Wiley and Sons, Inc., New York, 331 pps. $7.50

Dr. Roger G. Bates, chemist at the National Bureau of Standards, has written this book to make some order out of the widespread confusion about the principles of *p*H determinations. The rapid advances in *p*H meters have overshadowed the basic principles and the meaning of the *p*H numbers which are so easily obtained and used.

The first six chapters of this book are devoted to the presentation of the theoretical and experimental basis for a practical electrometric scale of acidity and compares the various possible scales as to usefulness and validity. The author shows that, although the *p*H value is a thermodynamic quantity, the actual value and measurements depend upon certain basic assumptions. There is a full discussion of the standard *p*H scale and the National Bureau of Standards *p*H standards, which are rapidly being adopted throughout the world. Dr. Bates stresses the importance of accurate and reliable standard solutions and gives detailed instructions for the preparation of these solutions.

A separate chapter is devoted to a discussion of scales of acidity and basicity in nonaqueous solutions. The last four chapters discuss the many experimental and practical aspects of *p*H measurement. The instrumentation is presented fully and clearly. The selection and preparation of electrodes and salt bridges are described and errors pointed out, and the care and handling of the instruments are outlined. Complete chapters are devoted to the discussion of the glass electrode and to automatic *p*H control as used in industry.

This book is recommended as a natural adjunct to the laboratory *p*H meter.

# CLINICAL CHEMISTRY

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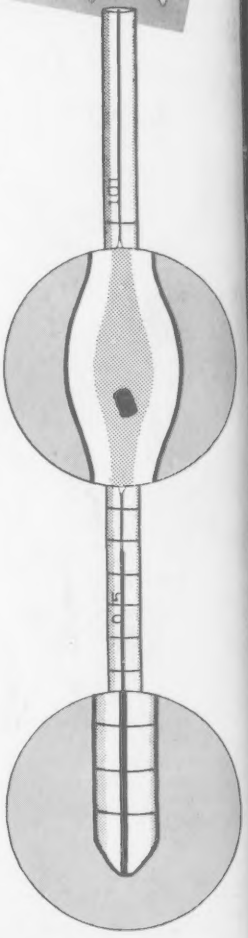


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